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The Effect of Naphthacene on the Fluorescence of Hydrocarbons*

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Fluorometric methods of analysis have been criticized on the ground that fluorescence is often too sensitive to contaminants to be reliable (2, 14). Thus Winterstein and his associates (17) reported that the blue fluorescence of pure anthracene or of chrysene is completely destroyed by as little as 0.00003 per cent of naphthacene (2,3-benzanthracene), found in crude anthracene preparations. Presumably this observation was made on solids. Sannié (14) cited Winterstein's results, and concluded that fluorometric methods are unreliable in determining the fate of carcinogenic hydrocarbons in tissue. Chalmers (6) stated that "Substances, such as naphthacene, may interfere with spectrum analysis made during extraction of anthracene or 3,4-benzpyrene from coal tar . . . . but, presumably, such substances are not present in normal tissues." Fieser (8), referring to Winterstein's work, stated that "The beautiful fluorescence of dilute solutions of the pure hydrocarbon (anthracene) is completely obliterated by 1/30,000 per cent of naphthacene." Bowen (4) reported that the blue fluorescence of pure solid anthracene is replaced by a strong greenish yellow fluorescence when naphthacene is present, and concluded that "this fluorescence is stimulated by light absorbed by the anthracene while the blue anthracene fluorescence is almost entirely suppressed."

The foregoing all suggested that naphthacene might be a specific quencher of the fluorescence of the carcinogenic hydrocarbons and thus be of use in their determination in animal tissues. Accordingly the fluorescence of six representative hydrocarbons was measured in liquid solution and in the solid state, and the effect of naphthacene determined in each case. While naphthacene proved to be without value as a specific inhibitor in these experiments, it also became evident that the "naphthacene effect" did not present any real obstacle to the use of fluorometric methods in the determination of hydrocarbons. Others have also concluded that fluorescence spectroscopy can be of value in such determinations (3, 10, 11).

METHOD

Preparation of hydrocarbon solutions.—The hydrocarbons included three potent carcinogens, 9,10-dimethyl-1,2-benzanthracene (DMBA),[1] 20-methylcholanthrene (MC), and 3,4-benzpyrene (BP); one weak carcinogen, 1,2,5,6-dibenzanthracene (DBA); one borderline compound, 1,2-benzanthracene (BA); and two noncarcinogenic hydrocarbons, anthracene (A) and naphthacene (NA). As obtained commercially none of them was pure; all contained substances that could be removed by chromatographic adsorption. For this purpose petroleum ether (Skelly solve B, b.p. 66–68° C.), acetone, and thiophene-free benzene, were purified by distillation in an all-glass still. Twenty-five to 50 mgm. of the hydrocarbons were dissolved in 150 cc. of solvent. The solvent used for dibenzanthracene and naphthacene was a 4:1 mixture of benzene and petroleum ether; for all others petroleum ether alone. The chromatograms were prepared in glass tubes, 20 × 1 cm., constricted at one end and plugged with fiberglas. A thick suspension of 100 mesh Al_2O_3 (Merck, activated at 230° C. for 3 hours) in petroleum ether was poured into the tube and suction applied, until a 10 cm. column had been deposited. The solution of hydrocarbon was then drawn through the column, and the chromatogram developed with petroleum ether. All operations involving hydrocarbons

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[1] The 9,10-dimethyl-1,2-benzanthracene used in these experiments was prepared by Dr. E. A. Prill. We are indebted to Dr. H. P. Rusch, of McArdle Memorial Laboratory, for this preparation.

were carried out in subdued artificial light. The progress of the adsorption bands was observed periodically in ultraviolet light. Most impurities were found to remain near the top of the column; a few descended more slowly than the hydrocarbon being purified, and only once, in commercial BP, was a faster moving component observed. The purified hydrocarbon was taken to dryness *in vacuo* at 40° C., dissolved in acetone, filtered, and water added to the warmed solution to a point of temporary cloudiness. Slow cooling to 0° C. produced well formed crystals, which were dried *in vacuo* in the dark. One adsorption and two crystallizations usually yielded a product having the correct melting point and characteristic fluorescence (Table I).

TABLE I: THE MELTING POINTS AND FLUORESCENCE OF PURE HYDROCARBONS RELATED TO THE CARCINOGENS

Hydrocarbon	Melting point, corrected: Found, °C.	Melting point, corrected: Recorded, °C.	Color in: UV light	Color in: Daylight
DMBA	122.8-123.5	122.6-122.9 (7)	Blue fluorescence	Pale yellow
MC	179.5-181.0	179.8-180.4 (18)	Light blue "	" "
BP	179.0-180.2	179-180 (1)	" " "	" "
DBA	267.0-267.5	266.6-266.9 (7)	Blue "	White, weak blue fluorescence
BA	162.0-162.5	161.4-161.8 (7)	" "	" distinct " "
A	216.0-216.4	215.6-216.5 (7)	" "	" strong " "
NA	343.8-344.0	341.5-343.0 (7)	Weak yellow "	Golden yellow

Master solutions of the hydrocarbons were prepared at concentrations of 100 μgm. per cc. of aldehyde-free 95 per cent alcohol [2] (acetone for DBA and NA). Aliquots of these solutions were evaporated *in vacuo* at 50° C. in volumetric flasks and made up to volume in petroleum ether (acetone for DBA and NA) for routine use. All standard solutions were stored in glass-stoppered bottles in a cool dark place.

For the measurement of fluorescence, 1 to 2 cc. of the standard solutions were placed in fluorometer tubes (culture tubes, 150 × 19 mm.), a small boiling chip was added, and the solvent removed under reduced pressure on the steam bath. Since anthracene sublimes easily, its solution was evaporated at 45–50° C. Apparently no significant amount of hydrocarbon was adsorbed by the boiling chip since the size of the chip did not affect the values obtained in replicate tubes. Contact with rubber was avoided by the arrangement shown in Fig. 1. The small residues of hydrocarbon, often as little as 0.1 μgm., were then dissolved in known amounts of solvent. A solvent was not considered fluorometrically pure unless the fluorescence of 10 cc. of the pure solvent and of the solvent plus several drops of $C(NO_2)_4$ agreed to within 1 to 1.5 ammeter units. Tetranitromethane is a powerful general inhibitor of fluorescence (12). Redistillation from a clean all-glass apparatus was often all that was necessary for the purification of commercial samples of the solvents.[3]

Determination of fluorescence.—The intensity of the light emitted by the fluorescent solutions was measured in a Coleman electronic photofluorometer,[4] model 12. In this instrument light from a capillary mercury arc passes through a lens and filter into the

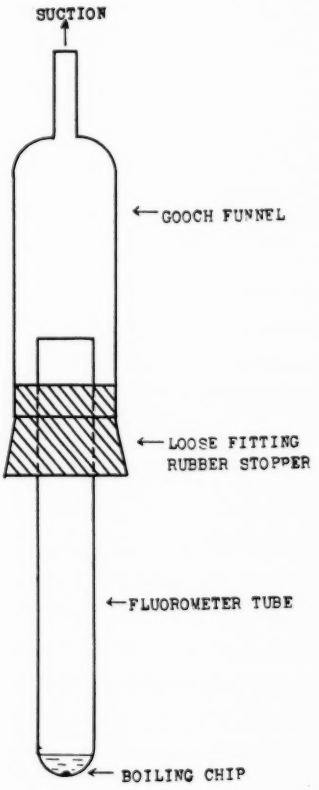

FIG. 1.—Arrangement for concentrating fluorescent solutions for quantitative determination.

[2] Aldehydes were removed by shaking for 2 to 3 hours with 10 gm. per liter of *m*-phenylenediamine, allowing to stand for 48 hours, and distilling from an all-glass still.

[3] Grossly contaminated solvents can be made fluorometrically pure by shaking with activated charcoal (norite A) and distilling. Thus when a liter of petroleum ether was shaken with about 50 gm. of charcoal and filtered, a product was obtained that on distillation gave no color with H_2SO_4 after hours of contact. The charcoal treatment, therefore, was definitely superior to the ordinary process of exhaustive extraction with H_2SO_4.

[4] We are indebted to Professor H. Steenbock for the use of this instrument.

tube of fluorescent solution. The emitted light leaves the tube at right angles, passes through a second filter, and strikes a photocell, the current from which is amplified and measured on a microammeter with an arbitrary scale of 100 divisions. The reading obtained for any given solution depends on the light source, the filters, and the amplification of the current generated. The filters used in the present experiments were a Coleman UV No. 1, which transmits the 365 mμ line of Hg, and the Coleman UV No. 2, which transmits in the region of 420 to 480 mμ. Since the intensity of the light source varied throughout each experiment, corresponding adjustments were made in the degree of amplification of the current generated. The fluorescence standard employed for this purpose was 0.0003 per cent quinine sulfate in 1 *N* H_2SO_4. This solution nearly duplicated the reading for 1 μgm. of 3,4-benzpyrene in 10 cc. of petroleum ether, for the particular system of filters employed. Several samples of the quinine solution were sealed in air in fluorometer tubes, either for routine use or for storage in the dark. Those in constant use decreased in intensity by about 8 per cent over a 2 month period; those in the dark remained completely stable as compared with the hydrocarbon standards. The intensity of the fluorescence of the hydrocarbons was expressed in the arbitrary 100 divisions of the ammeter scale,[5] with the instrument set so that 0.0003 per cent quinine sulfate gave a deflection of 50 units. The number of such "ammeter units" varied directly with concentration over a fairly wide range for each of the seven hydrocarbons studied (Fig. 2).

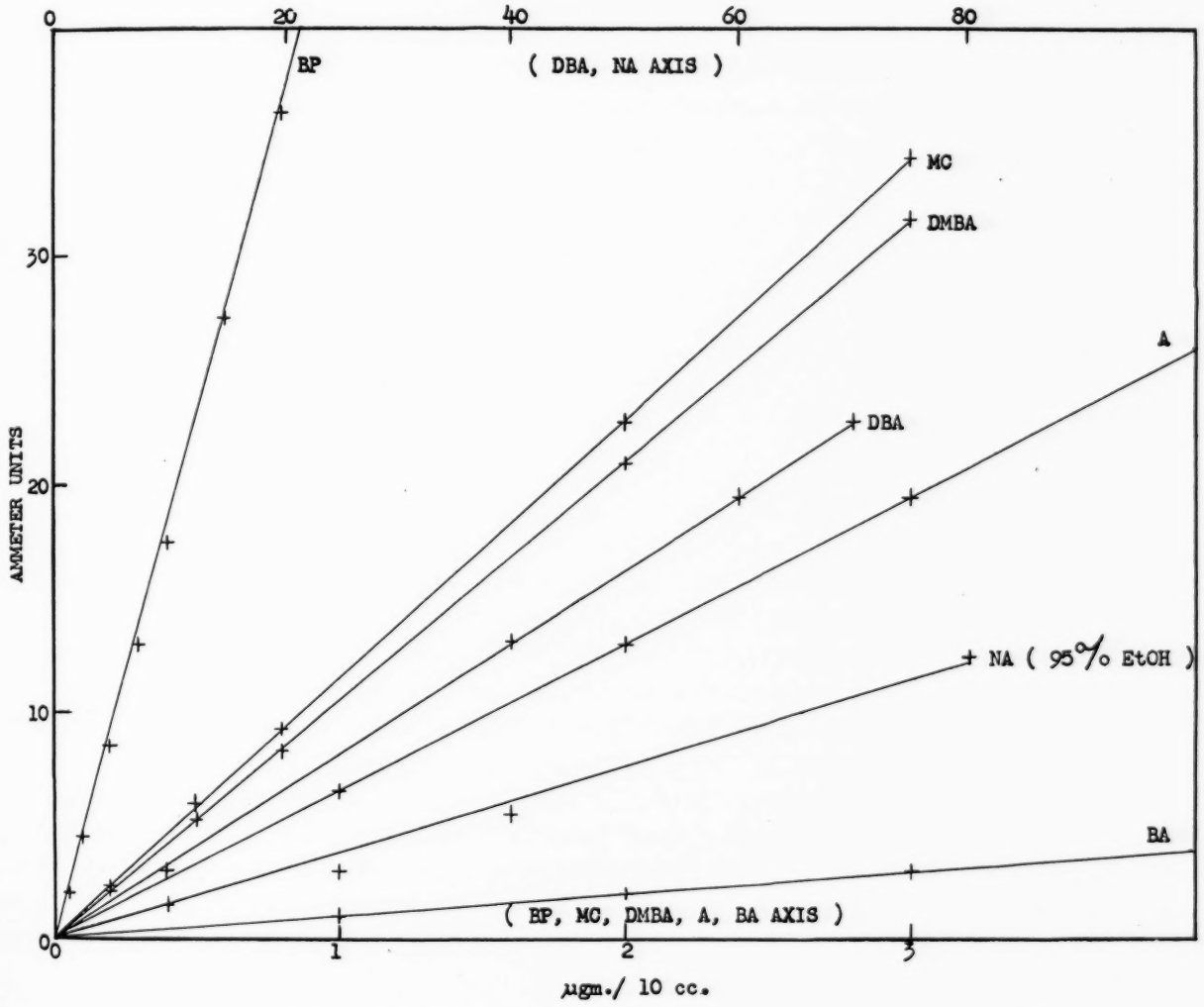

FIG. 2.—The variation of hydrocarbon fluorescence with concentration in petroleum ether solution.

Photography of fluorescence spectra.—The spectra of both solid and liquid solutions of the hydrocarbons were photographed through a large Bausch and Lomb

[5] A well defined unit of fluorescence would be highly desirable. Owing largely to technical difficulties the measurement of a fluorescence unit comparable to the extinction coefficient in use in absorption spectroscopy is not yet possible.

constant deviation spectrometer.[6] A cone of solid hydrocarbon was prepared by evaporating 1 to 2 cc. of an acetone solution containing 2 to 4 mgm. upon the inside surface of a tapered 15 cc. centrifuge tube, and the mouth of the tube was placed before the spectrometer slit so that the fluorescent cone covered it with a relatively even disk of light. Liquid solutions were placed in a cuvette made by bending an ordinary Nessler tube at right angles and fitting it with a parabolic aluminum reflector. A large perforated disk of black cardboard between the tube holder and the spectrometer prevented stray light from reaching the slit. Usually the width of the slit was 0.03 mm. and its height 0.5 to 1.0 cm. Fluorescence was excited by a Coleman U-11-M Universal ultraviolet Hg arc illuminator with a No. 1 filter transparent to the 3,650 Å line. Exposures were made on plates[7] particularly sensitive in the region 4,600–5,800 Å. Exposure times were determined by trial: 15 to 60 seconds were required for 2 mgm. films of the hydrocarbons when the slit width was 0.03 mm.; liquid solutions required 1 to 30 minutes of exposure depending on the concentration and solvent employed. Reference lines from a Hg arc were photographed on each plate. A wave length scale was made by photographing the 5,460 Å line of Hg through a Corning 5,400 Å filter, at settings of 50 to 100 Å intervals obtained by turning the wave length drum. The resulting scale corresponded almost exactly with spectra taken when the drum was set at 4,358 Å. Since the scale was literally photographed backwards it was most applicable to spectra taken on the opposite side of the plate.

EXPERIMENTAL

Naphthacene in liquid solution.—The effect of naphthacene on the fluorescence of solutions of the other hydrocarbons in petroleum ether is given in Table II. Contrary to the general statements in the literature, naphthacene was completely without effect as an inhibitor of the fluorescence of hydrocarbons in solution, even in concentrations equivalent to those of the fluorescing hydrocarbons themselves. Sometimes the fluorescence of the solution was increased by an amount equivalent to the fluorescence of the added naphthacene. The absence of any quenching effect was confirmed in four other solvents: pyridine, tetrahydrofurfuryl alcohol, ethanol, and acetic acid, and furthermore, naphthacene failed to alter the position of the bands in the fluorescence spectra of any of the solutions of hydrocarbon studied. Weigert (15) has also noted that naphthacene does not affect the fluorescence spectra of these hydrocarbons in acetone.

Table II: The Effect of Naphthacene on the Fluorescence of Solutions of Hydrocarbons in Petroleum Ether

Hydrocarbons, μgm. per 10 cc.	Fluorescence, "ammeter units"
10 A	57.0
10 A + 1 NA	57.0
10 A + 10 NA	55.0
1 BP	45.0
1 BP + 1 NA	45.0
1 BP + 10 NA	45.0
5 DMBA	53.0
5 DMBA + 10 NA	54.0
5 MC	48.5
5 MC + 10 NA	50.0
20 DBA	8.5
20 DBA + 20 NA	11.5
20 BA	23.0
20 BA + 20 NA	23.2
5 NA	2.0
20 NA	4.5

Table III: Fluorescence Spectra * of Solid Hydrocarbons in the Presence and Absence of Naphthacene †

	Pure hydrocarbon, Å	1:1,000 solid solution of N.A-HC, Å
Anthracene	4,225(150), 4,475(150), 4,775(150)	5,000(200), 5,300(200), 5,650(100) + bands of A
1,2-Benzanthracene	4,210(175), 4,475(175)	4,930(140), 5,290(375), 5,650(100) + bands of BA
1,2,5,6-Dibenzanthracene	4,300(100), 4,570(125)	4,960(175), 5,300(100), 5,650(100) + bands of DBA
3,4-Benzpyrene	4,300-5,000, densest at 4,400-4,600	5,000(200), 5,300(100), 5,650(100), no bands of BP
20-Methylcholanthrene	4,250-4,900, densest at 4,350-4,700	4,900-5,700, no MC bands
9,10-Dimethyl-1,2-benzanthracene	4,300-4,800, densest at 4,350-4,450	4,900(100), 5,225(150), 5,625(150) + bands of DMBA

* The centers and widths of the bands are indicated to ± 20 Å.

† With pure naphthacene (2,3–benzanthracene) a weak band at 5,550–5,700 Å was noticed on the plate. The spectrum is really continuous and extends to 6,500 Å (hand spectroscope).

Naphthacene in solid solution.—Solid solutions of naphthacene in other hydrocarbons were prepared by dissolving 1 part of naphthacene and 1,000 parts of other hydrocarbon in acetone and evaporating the mixture in a centrifuge tube. Photography of the fluorescent cone yielded the bands indicated in Table III. Naphthacene showed a weak band at 555 to 570 mμ;

[6] A small hand spectroscope (Zeiss direct vision with built-in wave length scale) was used to observe the fluorescence spectra of very dilute hydrocarbon solutions. This instrument absorbs little light and the spectrum may be observed directly while the solution is in the fluorometer.

[7] Eastman spectroscopic plates, type 103-G. We are indebted to Dr. W. F. Swann, of the Research Laboratories of Eastman Kodak Co., for these plates.

all the other hydrocarbons showed one or more strong bands in the range 410 to 480 mμ. (The optical setup was limited to the range 400 to 580 mμ.) Considerable variation was observed in the fluorescence spectra of the various pure hydrocarbons. In the presence of naphthacene, however, these variations decreased, and a new series of bands, characteristic of naphthacene in solid hydrocarbon solution, appeared. The usual maxima were at 495, 530, and 565 mμ. Even at the relatively high concentrations of naphthacene employed, the fluorescence of the original hydrocarbon persisted to a slight degree, perhaps owing to inequalities in the deposition of the solid mixture from solution.

It is possible that the "naphthacene effect" in solids is but a particularly intense example of a general phenomenon. Bowen (4) observed that small amounts of 2,3,6,7-dibenzanthracene or pentacene, a dark blue nonfluorescent compound, caused pure anthracene to fluoresce red. Preliminary experiments on solid solutions of anthracene or 1,2-benzanthracene in 3,4-benzpyrene, however, indicated that little if any changes in fluorescence occurred. In theory at least the naphthacene effect might extend to fluorescent compounds other than hydrocarbons and also to nonfluorescent compounds that absorb light (pentacene). The evidence, however, is meager: Ergosterol, with a weak blue fluorescence, retained the same fluorescence in solid solution with 0.1 per cent naphthacene. Cholesterol, which is nonfluorescent, also failed to fluoresce in the presence of naphthacene. The question also arises which compound, if either, in a solid solution of naphthacene and other hydrocarbon is responsible for the new fluorescence. Since naphthacene is fairly reactive chemically (9) the new fluorescence may be due to a naphthacene-hydrocarbon complex that is easily dissociated by solvents. However, the small percentage of naphthacene required ($<$0.1 per cent) argues somewhat against this suggestion.

The effect of naphthacene on the fluorescence of hydrocarbons in the liquid as contrasted with the solid state has been used unwittingly in this and other laboratories (5) as an indicator for the speed of solution of hydrocarbons applied to the skin of animals. Commercial samples of carcinogenic hydrocarbons usually fluoresce green to yellow in the solid state (presumably because of the presence of naphthacene or some similar contaminant), whereas in solution the impurity fails to affect fluorescence, and the true blue of the hydrocarbon itself appears. Thus, when impure hydrocarbon solution (blue) is applied to animal skin, the fluorescence changes to green as the solvent evaporates. Subsequently the fluorescence changes to blue, indicating that solution in the fat of the skin has occurred. However, when pure hydrocarbon is deposited on the skin of a normal rat or mouse, the treated area fluoresces only blue, whether the hydrocarbon is in solution or not. Naphthacene has also been employed as an indicator of the state of dispersion in aqueous colloidal solutions of various hydrocarbons (15).

Fluorescence of hydrocarbons in the molten state.—Pure molten anthracene cannot be excited to fluorescence (13). Similarly, upon fusion in air each of the solid hydrocarbons or its solid solution with naphthacene completely lost its ability to fluoresce and immediately regained its fluorescence upon resolidification. Solid BP and a solid solution of NA in BP exhibited the same changes when melted in a vacuum. However, a solution of BP in butyl cellosolve (20 μgm. per cc.) retained its fluorescence when heated to 180° C., the melting point of the hydrocarbon. Since the molalities of the BP in the two states differed by a factor of about 10^5, a "self-quenching" of fluorescence may have occurred in the molten hydrocarbon. Such quenching has been noted in hexane solutions of BP at concentrations above 20 μgm. per cc. (16) and of anthracene ($>10^{-3}$ molar) in benzene (13). If this were the full explanation, however, self-quenching should also be observed in the solid state.

SUMMARY

1. Details are given for the purification of carcinogenic and related hydrocarbons, for the quantitative determination of fluorescence in liquid solution, and for the photography of the fluorescence spectra of these hydrocarbons in solution and in the solid state.

2. Naphthacene (2, 3-benzanthracene), 0.1 per cent, in solid solution in 3,4-benzpyrene, 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, or in anthracene caused the appearance of bands in the fluorescence spectrum. These bands differed from those of either component and appeared to be characteristic of the fluorescence of naphthacene in solid hydrocarbon solution. The solid hydrocarbons and their solid solutions with naphthacene lost their ability to fluoresce upon fusion in air or *in vacuo* and immediately regained it on resolidification. 3-4-Benzpyrene when heated in liquid solution to its melting point retained its fluorescence.

3. When added to liquid solutions of the hydrocarbons, naphthacene was without effect as a fluorescence inhibitor. The so called "naphthacene effect" therefore offers no obstacle to the fluorometric determination of hydrocarbons in liquid solution.

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Factors That Alter the Fluorescence of Certain Carcinogens*

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The fate of carcinogenic hydrocarbons in the animal might be clarified considerably if analytical technics could determine the minimum amounts that are biologically active. Fluorescence is one property of sufficient intensity to meet this requirement; it has been used in preliminary metabolic studies (3, 4, 7, 8, 9, 17) and is approached only by the ability of certain of the hydrocarbons to absorb ultraviolet light. However, doubts have been expressed that a reliable fluorometric method for hydrocarbons is possible (2, 18), and there is a paucity of data suitable for use in evaluating fluorescence as a basis for the quantitative analysis of hydrocarbons in tissue. The present study deals with certain factors that alter the fluorescence of extracts from animal tissues and of seven representative hydrocarbons: 9,10-dimethyl-1,2-benzanthracene, 3,4-benzpyrene, 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene, 2,3-benzanthracene (naphthacene), and anthracene.

EXPERIMENTAL

The purification of the hydrocarbons, the methods of measuring their fluorescence in solution, and of photographing their fluorescence spectra have been described in the previous paper (16).

The fluorescence of various hydrocarbons.—A survey was made of the intensity of fluorescence of the 7 hydrocarbons in 37 fluorometrically pure (16) solvents (Table I). The two most obvious results encountered were (a) distinct variations in the intensity of the fluorescence of the various hydrocarbons, and (b) variations in intensity for any one hydrocarbon depending upon the solvent used. Of the hydrocarbons studied, naphthacene fluoresced the least; in most solvents its fluorescence was too weak for accurate measurement with the filters employed. Benzpyrene fluoresced most strongly, while the other hydrocarbons fell between, as follows: NA < DBA < BA < A < DMBA < MC < BP. The three most potent carcinogens in the series fluoresced the most strongly, but A, which is noncarcinogenic, and BA, which is practically devoid of activity, fluoresced more strongly than DBA, which is definitely carcinogenic. Evidently, therefore, the molecular characteristics that give rise to fluorescence must be other than those primarily concerned with carcinogenesis. The intensities of fluorescence of equimolar solutions of the hydrocarbons are given in Table II. In general the order of intensity was the same for all solvents yielding stable readings; *e.g.*, BP fluoresced 4 times as strongly as DMBA. However, MC fluoresced much more strongly in morpholine than its fluorescence in other solvents would have suggested; in most solvents MC fluoresced only 30 per cent as strongly as BP, but in morpholine it fluoresced 3 times as strongly as BP.

The fluorescence spectrum of each hydrocarbon was photographed at concentrations of 10 to 200 μgm. per cc. in pyridine and in petroleum ether (Skelly solve B, b.p. 66-68°C.) as described previously (16). While certain similarities were evident between these spectra, each hydrocarbon exhibited its characteristic fluorescence spectrum. In addition, certain regular variations with solvent were noted (Fig. 1). In petroleum ether the bands were fairly discrete, while in pyridine they were relatively broad and diffuse, and were shifted toward the red end of the spectrum.

Effect of solvents.—In CS_2 or aniline the fluorescence of all hydrocarbons was zero, and it was quite low in CCl_4, brombenzene, petroleum ether (Skelly solve B), and naphtha (Table I). Fluorescence was highest in the ethers tetrahydrofurfuryl alcohol, methyl cellosolve, butyl cellosolve, dioxane, and pyridine (a N-analogue of an ether). Other solvents were intermediate between these two groups. The variations in fluorescence did not appear to be associated with any one physical property of the solvent, although some regularity was observed within the few homologous series studied. In the primary normal alcohols fluorescence increased with increase in the molecular weight of the solvent, and in *n*-amyl acetate it exceeded that in ethyl acetate. Fluorescence in the *iso*-alcohols was slightly less than in the corresponding normal alcohols. However, fluorescence in acetic acid exceeded that in propionic acid, and in the series benzene,

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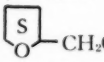
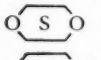
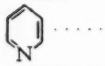
TABLE I: THE FLUORESCENCE OF HYDROCARBONS IN VARIOUS AERATED SOLVENTS

Ammeter Unit 0.000006 Per Cent Quinine Sulfate (0.0003 Per Cent ≅ 50 Units) *

Hydrocarbon Concentration, μgm. per 10 cc.	BP 1	MC 1	DMBA 1	A 5	DBA 20	BA 10	NA 50	Solvent blank
SSA (pentanes)	86.5	22.0	22.5	38.0	12.5	28.5	0.5	0.0
SSB (hexanes)	46.5	11.5	12.0	33.0	7.0	12.5	0.2	0.0
Naphtha	57.0	13.5	12.5	35.2	7.5	15.5	0.5	0.0
Benzene	120.0	40.0	29.5	58.0	21.0	64.0	1.3	0.0
Cyclohexane	88.0	20.0	19.5	42.5	10.0	25.0	0.7	0.0
Toluene	105.0	33.5	24.0	56.0	17.5	54.0	2.5	0.0
m-, *p*-Xylene	104.0	29.5	23.0	53.0	17.0	51.5	4.2	0.0
MeOH	79.0	27.5	21.0	41.5	14.0	26.5	9.0	1.0
EtOH, 95 per cent	120.0	36.5	27.5	45.0	17.0	40.0	10.5	0.0
EtOH, abs.	94.0	29.0	22.5	40.0	12.5	30.0	7.4	0.0
n-PrOH	118.0	25.0 †	25.0 †	40.5	16.0	41.0	7.2	0.0
n-BuOH	135.0	36.0	29.0	41.0	17.5	47.0	8.0	0.0
i-BuOH	129.0	34.0	27.5	43.5	17.0	42.0	7.0	0.0
n-AmOH	148.0	40.0	31.0	48.0	21.0	52.0	4.0	0.0
i-AmOH	145.0	37.0	32.0	49.0	18.5	46.0	4.0	2.0
ϕ-CH_2OH	80.0	30.0	22.5	43.5	23.5	61.0	25, 20, 40 ‡	1.5
	320.0	78.0	55.0	78.0	37.5	134.0	22.5	1.5
CH_2COOH	115.0	34.5	25.5	43.0	16.5	37.5	1.5	0.0
CH_3CH_2COOH	100.0	27.5	21.5	43.0	14.5	33.0	2.5	0.0
$(CH_3CO)_2O$	147.0	44.0	29.0	48.0	20.5	57.5	1.5	0.0
$Cl—CH_2CH_2—Cl$	146 †	40.0 †	29.0 †	32.0	15.0	52.0	0.0	0.0
$CHCl_3$	115 †	28.0 †	21.5	30.5	10.5	33.5	0.0	0.0
CCl_4	8 †	0.0	0.0	3.5 †	6.5	15.0 †	0.0	0.0
CCl_4, CS_2 free	9 †	0.0	2.0 †	4.0 †	7.0	14.5 †	0.0	0.0
ϕ-Br	28.0 †	14.0 †	13.0 †	13.0 †	6.0	13.0	6.3	0.0
$(Cl—CH_2CH_2)_2O$	240.0 †	38.0 †	44.0 †	56.0	42.0	120.0	15.5	19.0
Et_2O, abs.	95.0	22.5	20.5	43.0	15.0	30.0	4.5	0.0
$HOCH_2CH_2OMe$	235.0	55.0	38.0	54.5	27.0	84.5	2.5	0.0
$HOCH_2CH_2OBu$	186.0	49.5	36.0	53.0	25.0	76.5	2.0	0.0
$CH_3COOC_2H_5$	77.0	24.0	18.0	39.0	11.0	25.0	0.0	0.0
$CH_3COOn—Am$	100.0	27.0	21.5	43.0	13.5	31.5	1.0	0.5
	174.0	53.0	36.5	57.5	24.0	80.0	0.5	0.0
	14.0	45.0	18.0	4.5	13.0	6.0	0.0	0.0
	175.0	62.5	41.5	76.0	39.5	125.0	0.5	0.0
ϕ-NH_2, freshly distilled from Zn	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
CS_2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$(CH_3)_2CO$	86.0	27.5	19.5	41.0	11.5	31.5	1.7	0.0

* Values below 100 are recorded as observed; values above 100 are calculated from readings taken at lower concentrations of hydrocarbon.

† Fluorescence decreases on continued illumination.

‡ Value repeatedly equalled 25 when shaken, then fell to 20, and finally rose to 40 on continued illumination. Fluorescent cloud (invisible in daylight) formed while illuminated. It formed only in the UV beam and could be turned to one side by slowly rotating the tube.

TABLE II: COMPARATIVE FLUORESCENCE INTENSITY OF HYDROCARBONS

Hydrocarbon	Molecular weight	Micromolarity of solution used	Fluorometer readings per micromolar solution: Petroleum ether (66–67°)	Fluorometer readings per micromolar solution: Pyridine
Benzpyrene	252	0.397	117.0	441.0
Methylcholanthrene	270	0.371	31.0	168.5
Dimethylbenzanthracene	256	0.391	30.7	106.1
Anthracene	170	2.94	12.2	25.9
Benzanthracene	220	4.55	2.75	27.5
Dibenzanthracene	260	7.80	0.90	5.07
Naphthacene	220	22.7	0.009	0.023

toluene, and xylene fluorescence likewise decreased as the series ascended. Within the paraffin hydrocarbons, no regularity was evident; fluorescence was less in Skelly solve B (mainly hexanes) than in Skelly solve A (pentanes) although a further increase in molecular weight (naphtha fraction) resulted in increased fluorescence (Table I).

have given the lower reading in the fluorometer had the difference been due to a "filter effect" alone.

When water was added to a solution of hydrocarbon in a miscible solvent such as ethanol, a decided increase in the intensity of fluorescence resulted. The fluorescence of benzpyrene was 3 times as intense in an aqueous mixture containing 37 per cent of alcohol as in

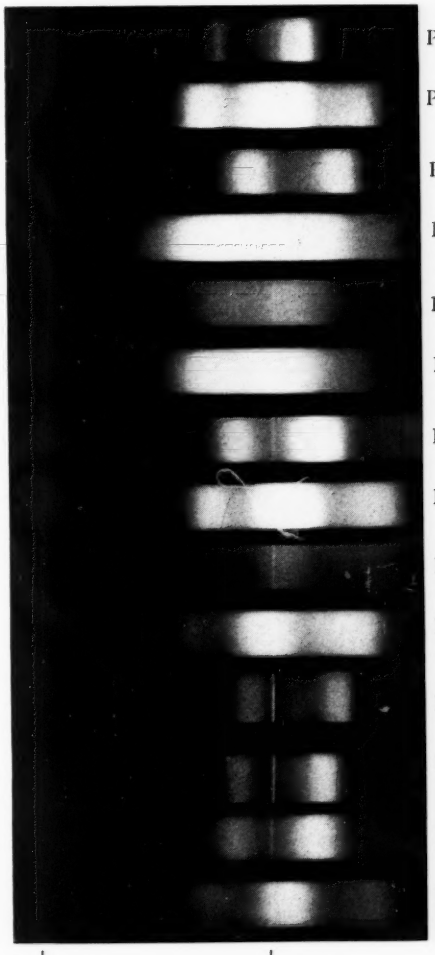


FIG. 1.—Fluorescence spectra of the hydrocarbons in petroleum ether and in pyridine.
P.E. = Petroleum ether. Pyr. = Pyridine.

Photographs of the fluorescence spectra indicated the reality of the increased fluorescence of hydrocarbons in pyridine as compared to petroleum ether. The fluorometer readings had depended, among other things, on the specific filters employed in the instrument, and while the band of light transmitted was relatively broad it was nevertheless conceivable that the differences noted between solvents might have been due to a shift in fluorescence spectrum out of the range of the transmitting filter. Actually, however, the shift in the bands was slight and such that pyridine should

absolute ethanol. Apparently this difference was due partly to an inhibitory effect of dissolved oxygen on fluorescence (5, 6, 20), since fluorescence was greatest at those concentrations of alcohol at which the solubility of O_2 was the least (Fig. 2). Similar fluorescence curves were obtained when water was added to solutions of benzpyrene in tetrahydrofurfuryl alcohol, acetone, pyridine, methyl cellosolve, and acetic acid. However, these curves indicate that difference in oxygen solubility was not the sole cause of the observed change in fluorescence. At the extremes of the con-

centration range the rates of change of O_2 solubility with percentage of solvent were either too small or too large in comparison with the corresponding changes in fluorescence. Oxygen is considerably more soluble (5 to 20 times) in most organic solvents than in water (13), and our experiments were performed with solvents that were in equilibrium with air under average conditions of 740 mm. Hg and 25° C. In such solvents, ether (Skelly solve B) and pyridine were irradiated in stoppered fluorometer tubes (ordinary glass, transmitting light above 320 mμ) at a distance of 38 cm. from a Cooper-Hewitt mercury arc. The decreasing intensity of the fluorescence over a 5 hour period is indicated in Table III. Every hydrocarbon except DBA was partially destroyed by the ultraviolet light. The unique stability of DBA to light in all the solvents

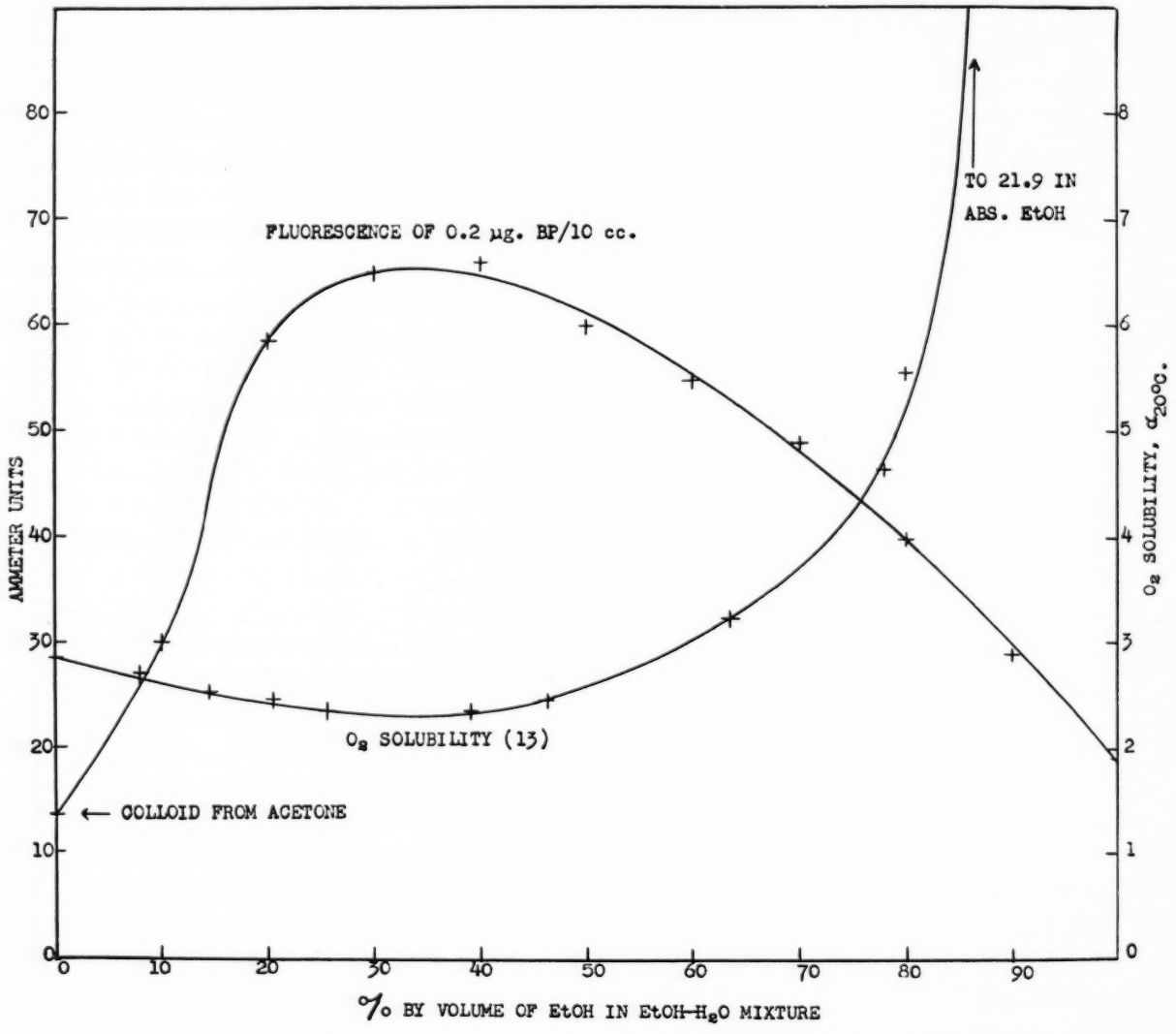


FIG. 2.—The fluorescence of benzpyrene and O_2 solubility in ethanol-water mixtures.

with the exception of those containing halogen, the fluorescence readings of these hydrocarbons were strictly reproducible.

Stability of hydrocarbons.—In most solvents fluorescence was stable for at least 1 to 2 minutes of continuous irradiation, but in halogenated solvents the initial intensity persisted for only a few seconds, after which the reading fell steadily. In many nonhalogenated solvents some destruction occurred upon prolonged exposure to strong ultraviolet light. Standard solutions of seven of these hydrocarbons in petroleum tried (Table I) parallels its great chemical stability (11).

The hydrocarbons (except NA) were refluxed for 1 hour in 10 per cent KOH in 80 per cent ethanol. The quantities employed varied from 0.2 μgm for BP to 40 μgm. for DBA. After having been refluxed, each hydrocarbon was quantitatively extracted with petroleum ether and measured fluorometrically. In every case destruction was less than the experimental error of 1 to 2 per cent.

Inhibitors of fluorescence.—The most powerful in-

hibitor encountered was tetranitromethane, which completely destroyed the fluorescence of the hydrocarbons in all solvents when the mixture was exposed to light. This compound was prepared (10) as follows: To 31 gm. of ice-cold fuming HNO_3 (d. 1.53) 50 gm. of ice-cold acetic anhydride were added gradually in 2 cc. portions and the temperature was kept below 20-25°C. The mixture was allowed to stand at room temperature for 1 week and was then poured into 200 cc. of water. The oily layer that separated was washed several times with water, and with a dilute solution of $NaHCO_3$, and was dried over Na_2SO_4. The final product was a faintly yellow liquid with a strong odor of nitric acid. Heating in the presence of an organic solvent was avoided, since $C(NO_2)_4$ is said to be dangerously explosive when so treated (1, 15). As little as 0.2 mgm. benzanthracene did not fluoresce in solution or in the solid state. Since $C(NO_2)_4$ is an unusual reagent in that it can nitrate organic molecules in neutral solution (19) it may actually have nitrated the benzpyrene. Tetranitromethane has been used as a diagnostic reagent in testing for unsaturation in organic molecules (12). In relatively concentrated solutions of the seven hydrocarbons (about 10 μgm. per cc.) a few drops of the reagent produced a brown color, which, however, was too weak in the desired range of 0.1 to 0.01 μgm. per cc. to serve as the basis for a colorimetric method of determination. Furthermore, color formation was non-specific since the unsaponifiable matter from tissue also yielded a brown color.

Benzpyrene did not fluoresce when dissolved in two other nitro compounds, nitromethane and nitrobenzene, nor in aniline, benzaldehyde, or carbon disulfide. The inhibition exhibited by these reagents was nonspecific. The inhibition of fluorescence by carbon disulfide was easily and completely reversible. When a solution of benzpyrene in this solvent was evaporated the residue yielded the correct amount of fluorescence in petroleum ether. Highly purified CS_2 (21) inhibited fluorescence completely.

TABLE III: THE STABILITY OF HYDROCARBONS TO ULTRAVIOLET LIGHT ($\lambda > 320$ mμ)

Fluorometer Readings in Petroleum Ether (Skelly Solve B)

Hydrocarbon	BP	MC	DMBA	BA	A	DBA
μgm. per 10 cc.	1	3	3	20	5	40
EXPOSURE TIME						
0 hr.	46.5	31.5	32.0	21.5	24.5	12.5
12 hrs. in dark	47.0	31.0	31.5	21.0	24.0	12.0
½ hr. to UV	43.5	22.0	26.0	21.0	23.0	12.0
1 " " "	41.0	19.5	23.5	21.0	23.5	12.5
2 hrs. " "	40.5	16.0	16.5	21.0	23.5	12.5
5 " " "	35.0	10.0	16.0	20.0	22.0	12.5
Readings in Pyridine						
μgm. per 10 cc.	0.3	1	1	5	5	20
EXPOSURE TIME						
0 hr.	57.5	62.0	39.0	60.0	61.0	37.5
12 hrs. in dark	58.0	63.0	36.5	61.0	61.0	38.5
½ hr. to UV	56.0	53.0	26.5	57.5	57.5	37.5
1 " " "	53.5	44.0	22.0	56.5	57.0	38.0
2 hrs. " "	53.5	36.5	21.0	55.0	57.0	38.0
5 " " "	51.0	19.5	9.0	50.0	50.0	38.0

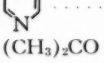
completely inhibited the fluorescence of 1 μgm. of benzpyrene in 10 cc. of petroleum ether. Its action was general; it also inhibited the fluorescence of riboflavin in pyridine, of thiochrome in *n*-butyl alcohol, of vitamin A in petroleum ether, and of fluorescent compounds in unsaponifiable matter from tissue, of paraffin oils, and of impure solvents. The latter property makes $C(NO_2)_4$ useful in determining the purity of solvents intended for fluorometric work (16).

The effect of the inhibitor was permanent. When excess $C(NO_2)_4$ was removed from a mixture of BP and $C(NO_2)_4$ by prolonged evaporation *in vacuo,* the residue did not fluoresce as such, nor when it was redissolved in petroleum ether. However, additional benzpyrene added to this solution showed the usual amount of fluorescence. Thus it appeared that a firm complex of the hydrocarbon and the $C(NO_2)_4$ had been formed. Jones (14) noted that 10-nitro-1,2-

Fluorescence of animal material.—The unsaponifiable matter from mouse tissue fluoresced much more weakly than the carcinogenic hydrocarbons and this fluorescence appeared to be general throughout the region of visible wave lengths. With the system of filters used in the determination of the hydrocarbons (16), the amount of light per unit weight emitted by mouse unsaponifiable matter was only 1/60,000 of that produced by the hydrocarbons: in petroleum ether (Skelly solve B) 20 mgm. of unsaponifiable

matter from the carcass per 10 cc. gave a reading of 15 ammeter units (Table IV) as compared to a reading of 46.5 for 1 μgm. of BP in this amount of solvent (Table I). When other solvents were employed, this discrepancy became even more evident, since the fluorescence of the animal material did not vary appreciably with solvent; its fluorescence was essentially the same in pyridine or tetrahydrofurfuryl alcohol as in petroleum ether or methyl alcohol (Table IV) thus sharply differentiating the fluorescence of the animal matter from that of the carcinogenic hydrocarbons.

TABLE IV: THE EFFECT OF SOLVENT ON THE FLUORESCENCE OF NONSAPONIFIABLE MATTER FROM MOUSE TISSUE

	Carcass	Liver	Organs	GI tract
Wet weight (= ½ mouse), gm.	3.82	0.34	0.28	0.64
Weight, nonsaponifiable matter, mgm. per 10 cc. solvent	20.0	3.4	3.0	1.2
Nonsaponifiable matter, per cent	0.52	1.00	1.07	0.19
Ammeter Units (SSB = 0, 0.00003 Per Cent Quinine SO_4 = 50)				
Skelly solve B	15.5	8.0	6.0	4.0
Benzene	18.0	12.0	5.3	4.7
Cyclohexane	18.0	8.5	4.0	4.0
Toluene	16.0	12.0	4.0	3.1
m-, *p*-Xylene	15.0	12.0	6.5	6.0
NeOH	15.0	5.7	4.5	4.5
EtOH, 95 per cent	16.5	11.5	7.5	6.0
EtOH, abs.	8.5	10.0	4.8	4.5
ϕ-CH_2OH	11.0	4.5	4.0	3.5
 —CH_2OH	14.0	9.0	5.0	5.0
CH_3COOH	17.5	7.0 *	6.0	4.0
CH_3CH_2COOH	16.0	9.0 *	6.5	4.0
$(CH_3CO)_2O$	14.0	6.0	5.0	4.5
$CHCl_3$	11.0	7.5 *	9.0	3.0
$HOCH_2CH_2OMe$	12.7	9.5	6.0	6.0
$HOCH_2CH_2OBu$	12.0	12.3	9.0	4.7
$CH_3COOC_2H_5$	11.0	7.0	2.0	4.0
	19.0	11.5	3.0	5.0
	18.0	9.0	3.5	5.5
$(CH_3)_2CO$	14.5	6.0	3.0	4.5

* Fluorescence decreases on continued illumination.

Furthermore the fluorescence of unsaponifiable matter was not enhanced by the addition of water. The latter experiments, however, were necessarily limited in scope since solid matter readily precipitated from solution when water was added. Like the fluorescence of the hydrocarbons the fluorescence of the unsaponifiable matter was unstable in chlorinated solvents, and nonexistent in CS_2 or aniline. On a weight basis, the unsaponifiable matter from liver or the other internal organs fluoresced about 3 to 5 times as strongly as material from the rest of the body.

The unsaponifiable matter from mouse tissue did not inhibit the fluorescence of the hydrocarbons (NA not tried) in solution since exact additivity of fluorescence was observed for each when added to petroleum ether (Skelly solve B) or pyridine solutions of unsaponifiable matter.

SUMMARY

1. The intensity of fluorescence in solution was measured for 7 hydrocarbons in 37 solvents. In most solvents fluorescence increased progressively in the following order: naphthacene < 1,2,5,6-dibenzanthracene < 1,2-benzanthracene < anthracene < 9,10-dimethyl-1,2-benzanthracene < 20-methylcholanthrene < 3,4-benzpyrene.

2. The fluorescence of each hydrocarbon was dependent upon the aerated solvent in which it was dissolved. Fluorescence was most intense in tetrahydrofurfuryl alcohol, pyridine, methyl cellosolve, and dioxane; it was low in the lower alkanes, and it was zero in CS_2 or aniline. In other solvents intermediate values were obtained. Stable, reproducible values resulted, except in halogen-containing solvents, in which fluorescence decreased rapidly on illumination.

3. With the exception of 1,2,5,6-dibenzanthracene the fluorescence of these hydrocarbons was partially destroyed in solution by prolonged exposure to strong ultraviolet light ($\lambda > 320$ mμ). Each hydrocarbon was stable to refluxing in 10 per cent alcoholic KOH for 1 hour.

4. The most potent inhibitor of fluorescence encountered was tetranitromethane; other inhibitors studied included nitromethane, nitrobenzene, benzaldehyde, aniline, and carbon disulfide. The inhibition by tetranitromethane was irreversible; the inhibition due to CS_2 was readily reversible. $C(NO_2)_4$ inhibited the fluorescence of all compounds studied: hydrocarbons, riboflavin, thiochrome, vitamin A, tissue substances, and impurities in paraffin or in ordinary solvents. It can be used in determining the "fluorometric purity" of a solvent.

5. The fluorescence of the unsaponifiable matter of mouse tissue was measured in eighteen solvents; on a weight basis the fluorescence of these extracts was very weak as compared with that of the carcinogenic hydrocarbons. The fluorescence of the unsaponifiable matter did not vary greatly with solvent and in mixtures it was strictly additive to the fluorescence of the hydrocarbons.

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Liver Tumors Following Cirrhosis Caused by Selenium in Rats

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It is generally believed that carcinoma of the human liver is frequently preceded by cirrhosis (4). In the case of experimental tumors of the liver, such as those produced by feeding *p*-dimethylaminoazobenzene [1] (1) to rats or feeding buckwheat to mice (5) or by the oral administration of a dose of carbon tetrachloride 2 or 3 times weekly (2) to mice, the livers often develop a cirrhotic appearance more or less concurrently with the early stages of tumor formation, which often takes place from 2 to 6 months after feeding of the agent has been begun. To our knowledge no one has reported the development of tumor in an experimentally cirrhotic liver in which the cirrhosis has been present without tumor for a long time; Moon's exhaustive review (6) of experimental cirrhosis does not mention this.

In this paper we are reporting the development of hepatic cell adenoma and low grade carcinoma in rat livers, beginning 18 months after the rats were placed on a seleniferous diet; this diet, after a 3 month period of a subacute type of liver damage, produced a chronic nodular cirrhosis without necrosis.

MATERIAL

Seven groups of 18 rats each of our inbred Osborne-Mendel strain were fed selenium in organic combination in corn and wheat and in a mixed inorganic selenide [2] beginning at 3 weeks of age at levels of 5, 7, and 10 parts per million of diet. The primary purpose of the experiment was to determine the lower level of selenium necessary to produce chronic toxicity. Among the 53 rats fed the seleniferous diet for periods of at least 18 months, up to the end of the 24 month experimental period tumors of the liver, some as large as 3 cm. in diameter, diagnosed as hepatic cell adenoma and low grade hepatic cell carcinoma developed in 11, while 4 others had pronounced adenomatoid hepatic cell hyperplasia that could be interpreted as a transition to tumor. In the 73 rats that died or were sacrificed before 18 months there were no tumors and no advanced adenomatoid hyperplasia, although cirrhosis was fairly frequent (after 3 months).

The diet for the 126 experimental and 18 control rats contained 12 per cent protein and consisted of 49 per cent corn, 44 per cent wheat, 3 per cent yeast, and 1 per cent each of cod liver oil, calcium carbonate, sodium chloride, and dried whole liver; seleniferous and nonseleniferous grains were combined in the proper proportions to give the desired level of selenium, while for the group on the inorganic selenium the material was simply added to the diet composed of nonseleniferous materials. The rats were all females, which are slightly more susceptible to selenium toxicity than males (9, 11). Seventy-one of the 126 experimental rats and 14 of the 18 control rats were examined microscopically.

Survival Periods by Groups

Table I gives the number of rats in each group that died, or that were sacrificed because of poor condition, during various intervals of time.

On a dosage level of 10 parts per million of selenium obtained from corn and wheat, 25 of 36 rats died during the first 3 months and only 4 lived the 2 year experimental period, while on a level of 5 parts per million only 2 of the 36 died during the first 3 months and 19 lived the full 2 years, nearly as many as in the selenium control group and our various other control groups, in which about two-thirds survived this period. Survival on 7 parts per million from corn and wheat and 10 parts per million from the selenide was intermediate between the groups previously mentioned.

Description of Liver Damage by Selenium

Only a brief summary will be given here. The liver damage was of two types, depending on whether death occurred before or after 3 months on the seleniferous diet; in the period from 2 to 4 months features of both types were seen. Rats that died during the first 3 months, generally between the 3rd and 6th

[1] Although this dye is commonly called butter yellow it should be emphasized that it is not used as a food color.

[2] A solution of ammonium potassium sulfide and ammonium potassium selenide, containing 48 gm. of Se per liter of solution.

weeks, usually had bloody or occasionally clear yellow fluid in the abdomen, and dark red granular livers with a hemorrhagic appearance. Microscopically there was a subacute type of damage with varying degrees of hepatic cell necrosis, atrophy, hyperplasia, cystic dilatation of sinusoids, and focal myelosis. Fibrosis, pigmentation, and bile duct proliferation were not striking. The gross abdominal hemorrhage appeared to originate by rupture of distended subcapsular sinusoids; often there was an organizing, pericapsular hemorrhagic exudate. Periportal or other intrahepatic hemorrhage was negligible.

Most of the rats living through, and presumably not as much affected during, this first phase of selenium toxicity developed a chronic type of liver damage without intrahepatic hemorrhage or necrosis, with less of cystic sinusoids and myeloid foci, and with increasing portal fibrosis, distortion of architecture, focal capsular retraction, and focal hepatic cell hyperplasia. These changes expressed themselves as varying degrees of a nodular cirrhosis (Table II), which in its advanced stages is a portal or Laënnec's cirrhosis according to the criteria of Moon (6). Nodularity was sometimes so advanced that the smaller lobes resembled miniature bunches of grapes. Atrophy was relatively uniform among the different lobes, while regenerative hyperplasia and tumors tended to be more noteworthy in the left lateral and caudate lobes.

Hemosiderin pigmentation was usually slight. Ascites was occasionally, and some degree of hemoperitoneum rarely (3 times), present; the source of the hemoperitoneum was not determined.

Lesions caused by selenium in viscera other than the liver were not extensive and none was very characteristic. With the control group as a base line (and this showed few spontaneous lesions) there were slight hyperplasia and hemosiderosis of the splenic pulp, slight hyperplasia of the bone marrow, slight focal myocardial fibrosis, and minor renal changes. There were no gastrointestinal, pancreatic, pulmonary, adrenal, or gross cutaneous alterations, and osseous lesions were occasional and slight. In the younger rats there was slight Kupffer cell and splenic reticulum cell hyperplasia, and some testicular atrophy.

TABLE I: SURVIVAL PERIODS BY GROUPS

Level of selenium in diet	Period of survival in months					Number in group
	3 or less	3½–11½	12–17½	18–23½	24	
5 p.p.m. (corn)	2	1	5	1	9	18
5 p.p.m. (wheat)	0	1	3	4	10	18
7 p.p.m. (corn)	7	0	1	3	7	18
7 p.p.m. (wheat)	9	2	3	1	3	18
10 p.p.m. (corn)	13	0	0	2	3	18
10 p.p.m. (wheat)	12	1	2	2	1	18
10 p.p.m. (selenide)	2	6	3	1	6	18
Total experimental	45	11	17	14	39	126
Control	0	2	2	2	12	18

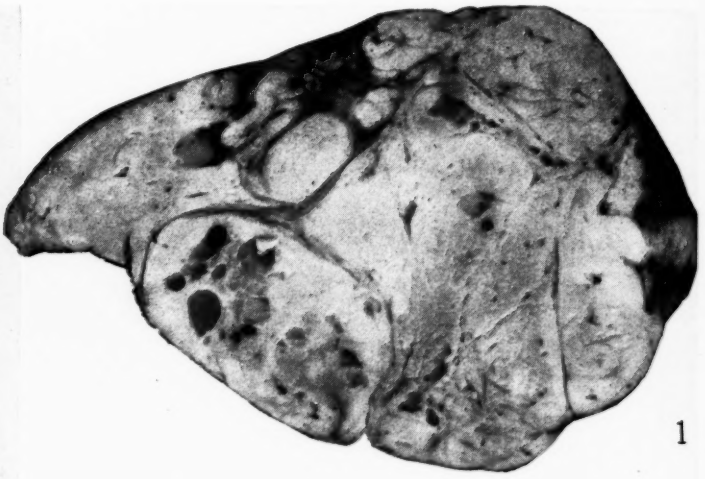
TABLE II: CIRRHOSIS IN RATS SURVIVING MORE THAN 3 MONTHS

Degree of cirrhosis	Months on experiment				Total
	3½–11½	12–17½	18–23½	24	
None	4	6	3	7	20
Slight	2	2	5	11	20
Moderate	3	2	2	9	16
Advanced	1	6	3	9	19
Extreme	0	0	1	3	4
Undetermined	1	1	0	0	2
Total	11	17	14	39	81

DESCRIPTION OF ADENOMA AND CARCINOMA IN CIRRHOTIC LIVERS

After ingesting the seleniferous diets for 18 months, 11 of the 53 surviving rats, 43 of which had cirrhosis, developed definite liver tumors up to 3 cm. in diameter, and 4 others advanced adenomatoid hyperplasia (Table III).

The table shows that the tumors were somewhat more frequent in the left lateral than in the other lobes of the liver, and that both single and multiple tumors occurred, the latter more frequently. There



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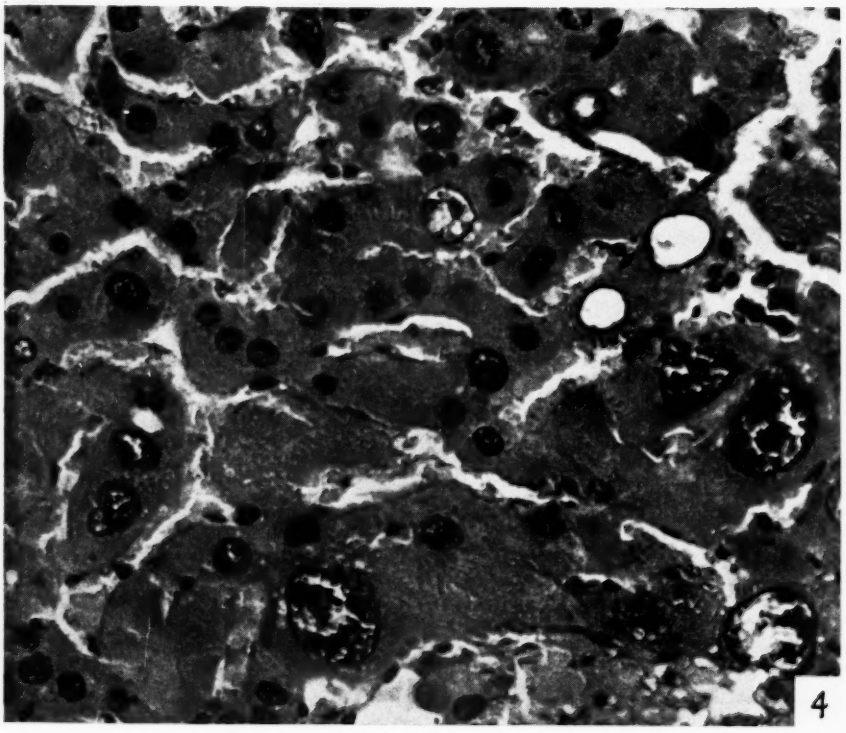

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FIGS. 1-4

was no parallelism between the degree of cirrhosis and the presence of tumor, except that there were no tumors in the 10 rats of this age group (18 to 24 months) without cirrhosis. The tumors were usually well circumscribed (Figs. 1, 2, 3) and paler and more granular than the surrounding liver tissues (after formalin fixation). Some of the larger ones had a moderately lobulated appearance (Fig. 1). No encapsulation was noted upon gross examination and no metastases were seen.

Upon microscopic examination, portions of the peripheries of the tumors were found separated from the rest of the liver by collagenous fibers, but there was no complete encapsulation. The growths were composed of fairly regular to irregular cords of large hepatic cells (Figs. 4 to 7), usually more oxyphilic than the surrounding liver in the adenomas and less oxyphilic in the carcinomas. Some tumors showed no mitoses after several minutes' search and in others a few or even a moderate number were seen in a shorter time. Bile duct proliferation was slight except in one instance (Path. 1550), where the peripheral 1 to 2 mm. of a 3 cm. tumor was composed of small bile ducts.

TABLE III: DATA ON LIVER TUMORS AND ADENOMATOID HYPERPLASIA IN RATS FED SELENIUM

Pathology No.	Months on experiment	Dosage, Se, p.p.m.	Cirrhosis, degree	Size and location of tumor	Microscopic diagnosis
1450	23	7 (wheat)	Slight	5 mm.; left lateral lobe	Adenoma
1498	24	10 (corn)	Advanced	8, 7, and 3 mm.; caudate lobe	Adenoma and low grade carcinoma
1520	24	10 (corn)	Advanced	Numerous scattered; to 7 mm.	Low grade carcinoma and adenomatoid hyperplasia
1524	24	5 (wheat)	Moderate	$3\frac{1}{2} \times 3\frac{1}{2} \times 3$ cm. in left lateral lobe; few elsewhere to 9 mm.	Adenoma
1549	24	7 (corn)	Advanced	$1.6 \times 1.6 \times 1.0$ cm.; left lateral lobe	Adenoma
1550	24	5 (corn)	Slight	$3.2 \times 2.7 \times 2.0$ cm., left lateral; few elsewhere to 4 mm.	Adenoma
1559	24	5 (wheat)	Slight	1.3 cm.; median lobe	Adenoma
1561	24	5 (corn)	Slight	Several up to 1 cm.; chiefly left lateral	Adenoma
1564	24	10 (selenide)	Advanced	About 10 up to $1\frac{1}{2}$ cm.; chiefly median and left lateral	Carcinoma, low grade
1565	24	10 (selenide)	Moderate	One dozen up to $1\frac{1}{2}$ cm.; right and left lateral	Carcinoma, low grade
1596	24	7 (corn)	Advanced	Multiple in left lateral lobe $3\frac{1}{2} \times 3 \times 2\frac{1}{2}$ cm.	Carcinoma, low grade
1006	18	10 (selenide)	Extreme		Adenomatoid hyperplasia
1201	$20\frac{1}{2}$	10 (corn)	Advanced		Adenomatoid hyperplasia
1543	24	7 (wheat)	Advanced		Adenomatoid hyperplasia
1560	24	7 (wheat)	Slight		Adenomatoid hyperplasia

Hemosiderin pigmentation and fibrosis within the tumors were not striking; focal necrosis was seen rarely, and hemorrhage not at all. Fatty degeneration was slight and was the same as, or a little less than, in the surrounding liver; the livers of the selenium control rats also showed a slight fatty degeneration, which was not found in our other control groups on more adequate diets. A few of the tumors enclosed small foci of myeloid cells, and in a few there were

DESCRIPTION OF FIGURES 1 TO 4

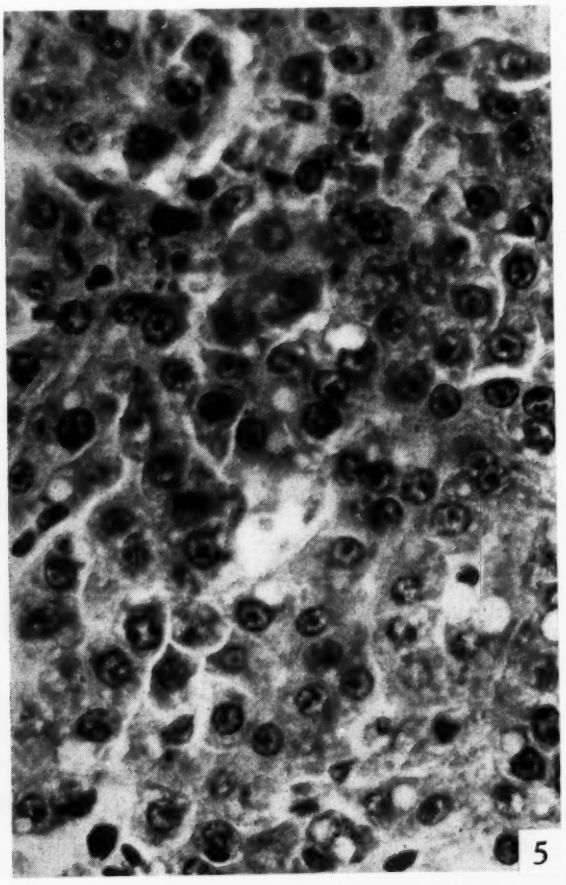
The photographs were made by Mr. M. L. Foubert of the U. S. Department of Agriculture and Mr. F. H. Meiller of the Food and Drug Administration.

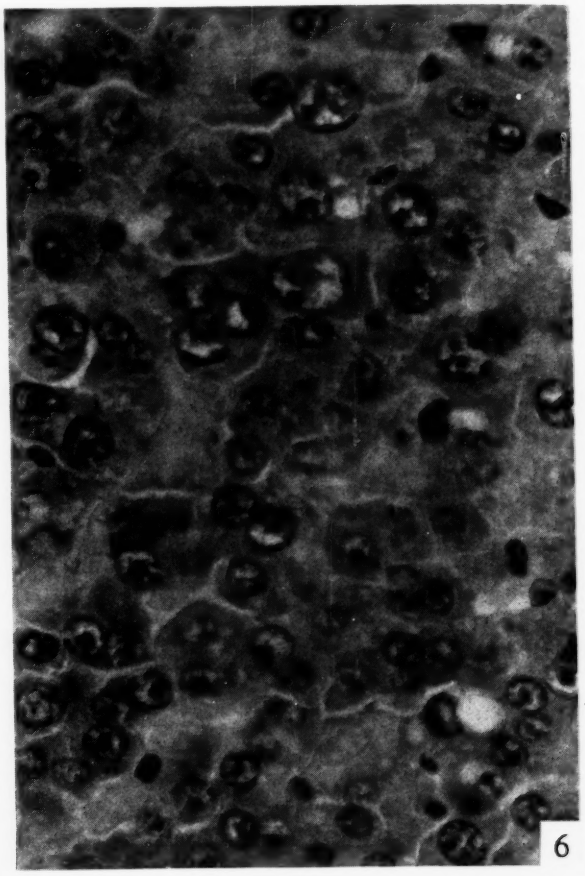
FIG. 1.—One of the larger tumors, in the left lateral lobe of rat 1524, showing moderate lobulation, small cystic areas in one lobule, and a portion of nonneoplastic liver to one side of the tumor. Mag. × 2.

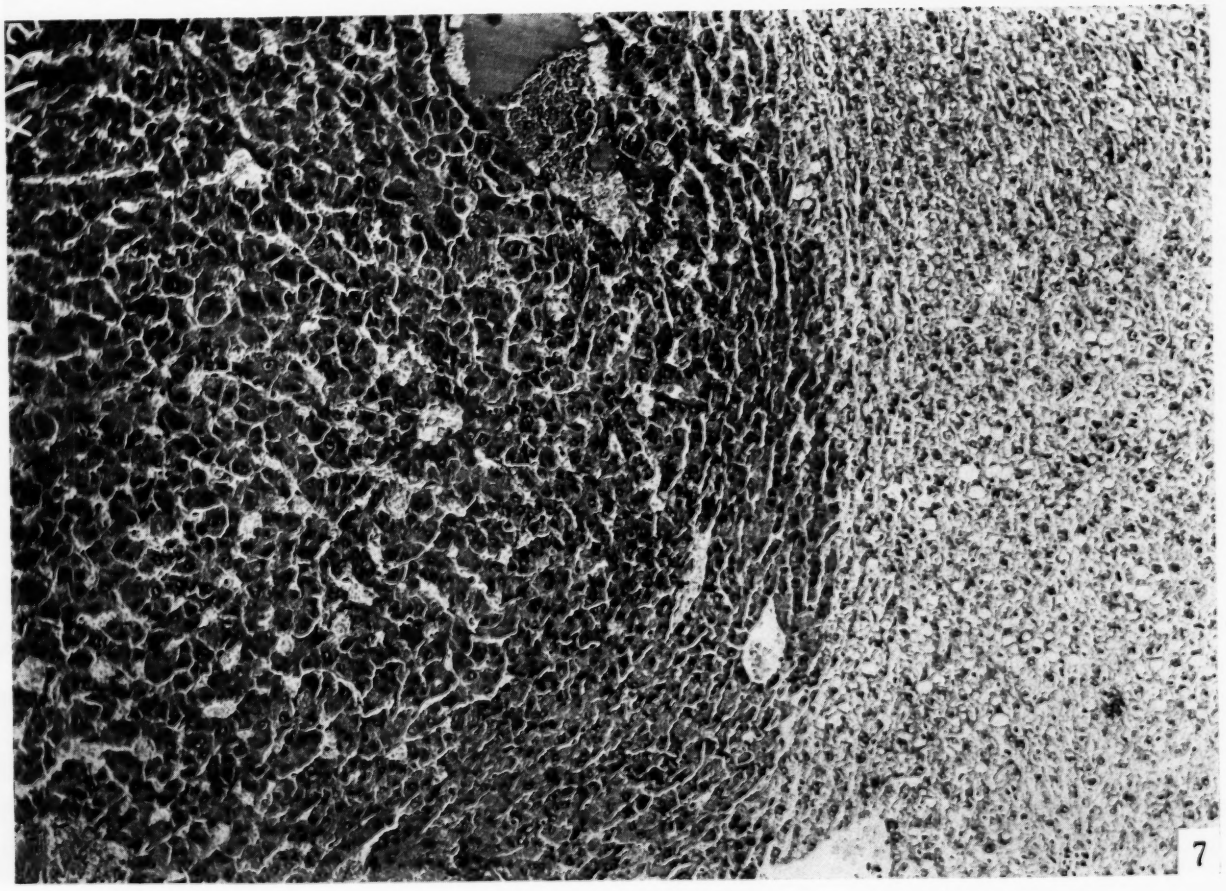
FIG. 2.—Two of the tumors in the left lateral lobe of the liver of rat 1596. Mag. × 8.

FIG. 3.—One of the tumors in the caudate lobe of the liver of rat 1498. Mag. × 8.

FIG. 4.—Area of adenoma in the median lobe of the liver of rat 1559, showing the short irregular cords of hepatic cells, with more variation in nuclear size than is usually seen, and a few vacuolated nuclei. Mag. × 125.







FIGS. 5-7

small cystic areas. Since sections were not made of every tumor, some livers listed as showing adenoma may have contained carcinoma also, and *vice versa*.

The differentiation between adenoma and low grade carcinoma was difficult to make in this series of tumors; the latter showed greater irregularity of liver cell cords, decreased oxyphilia of liver cells, more mitotic figures, and an invasive tendency at their margins. They were very similar to many of the low grade carcinomas of the rat liver seen after the ingestion of *o*-aminoazotoluene, and anyone who has

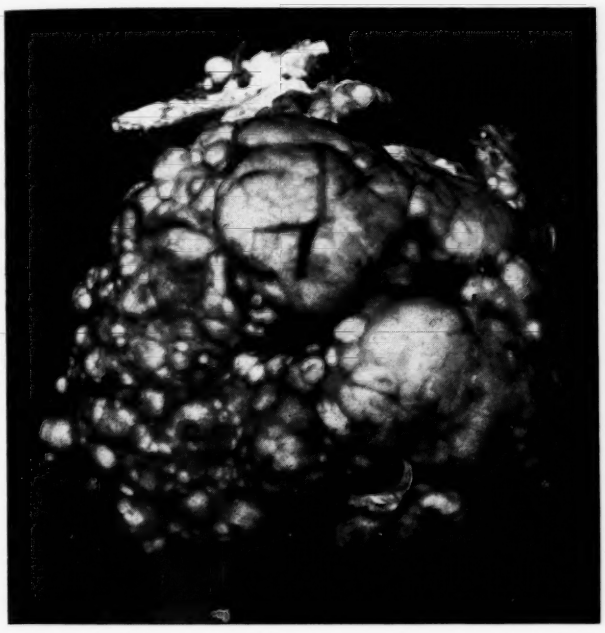


FIG. 8.—Liver of rat 1523, showing a high grade of nodular cirrhosis resulting from the feeding of 10 parts per million of selenium for 24 months. Mag. × 1½.

studied a series of the latter tumors will appreciate the difficulty of deciding just when the borderline between nonmalignant and malignant tumor has been passed, and also just when hyperplasia has passed into tumor. The differentiation into adenoma and low grade carcinoma was made chiefly because it was an orthodox procedure; perhaps the tumors could as well have been grouped under the term "hepatoma" or "hepatoma of low malignancy."

INCIDENCE OF SPONTANEOUS TUMORS OF THE LIVER

The incidence of spontaneous adenoma and low grade carcinoma of the liver in our control rats and in rats fed substances other than selenium was low; in 350 rats finishing a 2 year experimental or control period there have been 4 such tumors in 3 rats, in 200 rats from 18 to 24 months there has been one, and in nearly 1,000 younger rats none. These spontaneous growths were from 1 to 2 cm. in diameter and none had metastasized; there was no associated hepatic cirrhosis. The incidence of lymphosarcoma, leukemia, and spontaneous tumors of viscera other than the liver, for which careful records are kept, is not different in the rats reported upon in this paper from that in our entire group.

DISCUSSION

Liver damage in rats fed seleniferous grain was first reported by Franke (3) in 1934, although at that time it was not certain that selenium was the offending agent. For reviews of selenium toxicity in general and of the pathological manifestations of selenium poisoning, the reader is referred to the publications of Moxon (7), who carried on Franke's work after the latter's death, and of Smith (10).

As has been stated in the preceding tables and text, rats coming to autopsy at all ages up to 18 months had no advanced adenomatoid hyperplasia or tumors of the liver. In view of the relatively large percentage showing tumors at 24 months, which is the length of time chosen as the standard for ending our chronic toxicity experiments, there may be indicated the reaching of a "tumor age" or added susceptibility to certain carcinogenic influences at some time between 18 and 24 months for our colony of rats. This is very similar to the age condition necessary for the occurrence of spontaneous pulmonary lymphosarcoma (8) in our rats. Another possibility is that age as such is not a factor and that this length of time is required for selenium to produce its carcinogenic effect.

SUMMARY

Eleven of 53 rats developed adenoma or low grade carcinoma in cirrhotic livers, and 4 others advanced adenomatoid hyperplasia, after having survived for 18 to 24 months on diets containing 5, 7, or 10 parts per million of selenium; no tumors occurred in 73 rats surviving less than 18 months, although after 3 months cirrhosis was frequent. In control rats 18 to 24 months of age the incidence of spontaneous hepatic tumors was less than 1 per cent.

DESCRIPTION OF FIGURES 5-7

FIG. 5.—Low grade carcinoma in the liver of rat 1565. A greater departure from normal hepatic architecture is seen than in the adenoma shown in Fig. 4. Mag. × 512.

FIG. 6.—Low grade carcinoma in the liver of rat 1564. Mag. × 512.

FIG. 7.—Edge of the adenoma (darker portion of print) in the left lateral lobe of the liver of rat 1450. Mag. × 107.

This appears to be the first report of tumors arising in experimentally cirrhotic livers after the cirrhosis had been present without tumor for a relatively long period of time.

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Quantitative Determination of the Growth of a Transplantable Mouse Adenocarcinoma

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The growth of tumors as measured by volume has been reported by Chambers and Scott (2) for the Jensen rat sarcoma, by Konsuloff (3) for a mammary adenocarcinoma in mice, and more recently by Schrek (7) for various transplantable tumors. Their reports deal with tumors whose volumes are large enough to permit the determination of linear dimensions. In particular, Schrek (8) and Mayneord (4) have shown that the volume can be given in good approximation as equal to 0.5236 d^3, where d, the mean diameter, equals the cube root of the product of the three diameters measured at right angles to one another.

A plot of the logarithm of volume *versus* age from the data reported shows that for small volumes growth is a simple exponential function of time. At larger volumes, above 1 cc., the rate of growth diminishes. An obvious inference is that the diminution is probably due to lack of supply of nourishment to the tumor. Of greater interest however, is the growth of the tumor in the microscopic state, since an exponential law of growth implies that the major portion of the tumor life is spent in that condition. A replot of Schrek's (7) data on inoculum size *versus* latent period suggests that the early volume growth of rat tumors is exponential. This is suggested also by the data of Brues, Weiner, and Andervont (1) in their report on the latent period of chemically induced tumors in mice.

The purpose of the present investigation was to measure the rate of growth of a transplantable mouse adenocarcinoma, the Marsh-Simpson tumor, when inoculations of varying numbers of viable cells were made into mice of the Marsh-albino strain. The growth curve was plotted from the following data: (a) the volume distribution of cell sizes, (b) the average volume of inoculum with, (c) its latent period, and (d) the volume of the tumor at any given time after it had become palpable. These data gave four points on a curve describing the growth of the average tumor cell of this carcinoma. This paper is a preliminary report of the work to date.

EXPERIMENTAL PROCEDURE

Tissue was chosen from tumors that had not grown for more than 6 days after they had been palpated. Only sections of pearly white tissue were made into a suspension in Tyrode's solution, by manual grinding. The suspension was strained by filtering through a double layer of handkerchief linen. Microscopic examination of suspensions showed that the linen prevented clumps of cells or small pieces of tissue from passing into the filtrate.

The count of viable tumor cells was made according to the method described by Schrek (9). To 0.2 cc. of suspension were added 4.8 cc. of eosin stain dissolved in Tyrode's solution (1:2,000). The solution was well shaken for at least 2 minutes and the viable cells were counted in a counting chamber. Since the cells showed a tendency to sink to the bottom of the container, it was necessary to keep the suspension agitated at all times in order to insure uniform results.

Mice were inoculated with 0.05 cc. of cell suspension and palpated daily thereafter. The latent period was designated as the interval between the time of inoculation and the time when the tumor was definitely palpable. Two observers agreed that the tumor volume was of the order of 0.5 cu. mm. when first definitely palpable. This estimate was based on palpation of beads of known volume.

The data for the Marsh-Simpson tumor, showing the relation between the average latent period and the number of viable cells inoculated, are plotted in Fig. 1. Each point represents the average latent period for each emulsion. Inspection of the curve shows that the trend is logarithmic. The solid line is the curve that most nearly represents the average of the data. The dotted lines define the zone within which the scattered data lie. The intercept of the solid line with the ordinate axis is at 2×10^6 cells, which is the average number of cells in a tumor when it is palpable. Likewise the zone lines show the spread in the number of cells.

In order to determine to what extent logarithmic growth is followed, it is necessary to know the actual

volume of the cells in the inoculum and the volume of the tumor at various times after it has grown to a palpable size. These data combined with the latent period give three points on a graph of size *versus* time. The fourth point is the average volume of a single cell calculated from the measured diameters on the assumption that the cell is spherical. Accordingly, measurements were made of the volumes of tumors whose latent period and inoculum size were known, but the method was soon found impractical because of minute size of these nodules, their shape, and the existence of multiple tumors along the injection tract. Instead, the mouse was sacrificed and the tumor excised and placed in a pipette graduated in hundredths of a cubic centimeter. To facilitate insertion of the tumor, the end of the pipette was flared in a Bunsen flame by means of a carbon rod. Those tumors that were too large to measure conveniently in the pipette were measured in a graduated cylinder by the volume of water displaced.

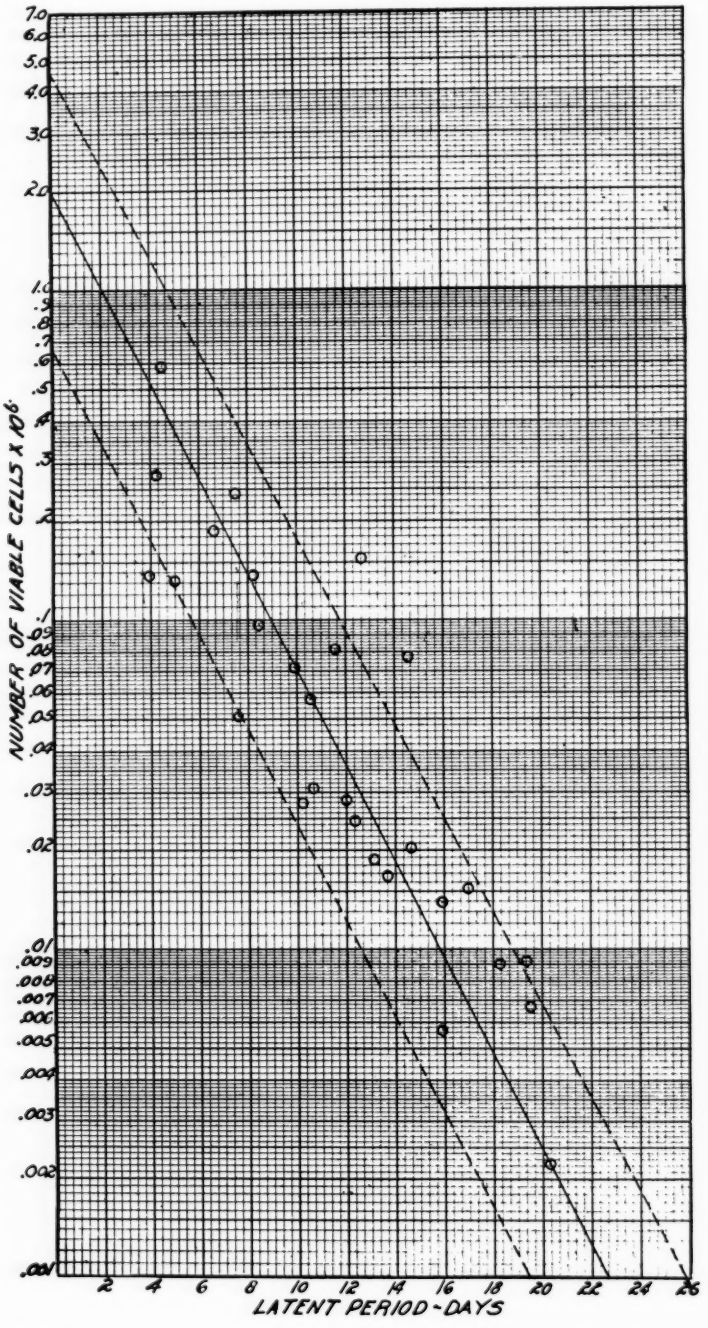

FIG. 1.—Relation between average latent period and number of viable cells inoculated.

To estimate the total volume of inoculated cells it was necessary to measure the average volume of the individual cells and then multiply by the number of cells in the inoculum. Approximately 1,000 cells were measured while in suspension in fairly low concentrations. Under these conditions the separate cells assumed a spherical shape. This measurement also gave time at which the tumor became palpable was taken as the origin of time ($t=0$) so that the latent period is negative while the time at sacrifice is positive. The tumor volume at the time of palpation is seen to be about 1×10^{-3} cc. This figure agrees with that from the latent period curve in Fig. 1 where the number of cells at $t=0$ was 2×10^{6}. When this figure is multi-

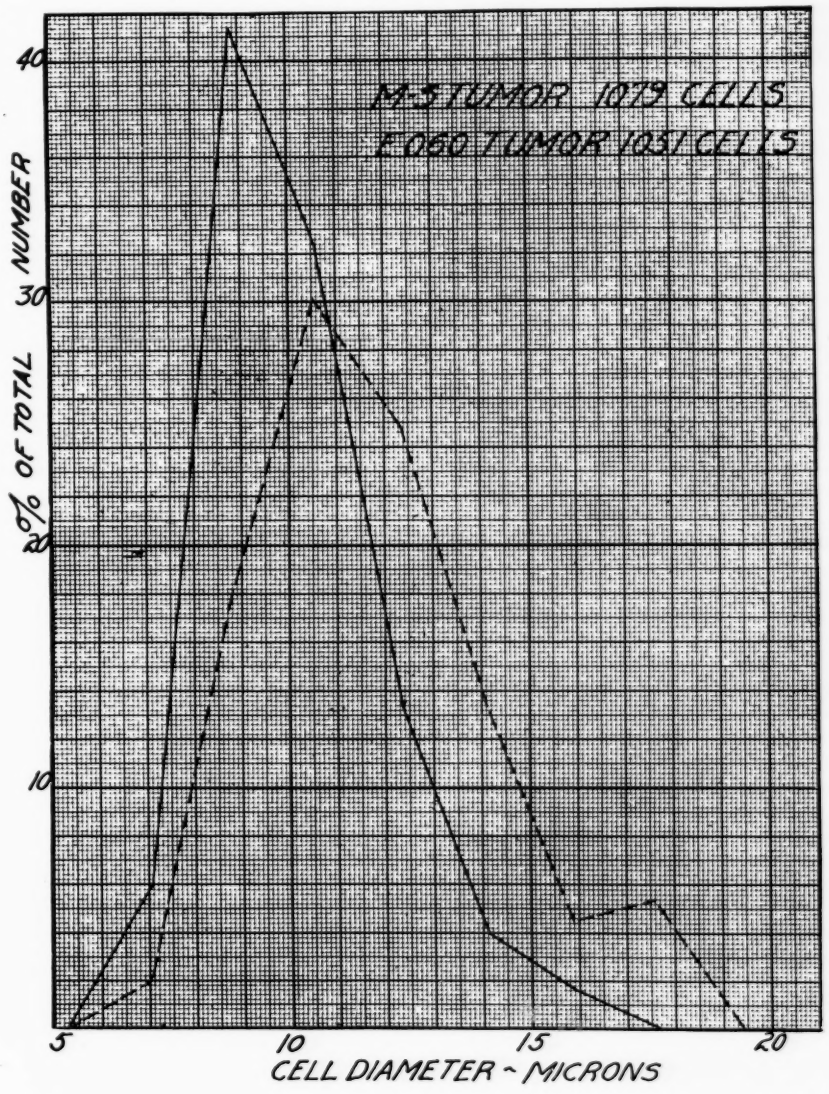

FIG. 2.—Distribution of cell diameters for Marsh-Simpson tumor and EO60 tumor.

the fourth point on the curve mentioned above. Fig. 2 shows the frequency distribution of the cell diameters as measured directly on the microscope. From these data it was found that the tumor cells had an average diameter of 10.2 micra and an average volume (assuming a spherical shape) of 5.5×10^{-10} cc. For comparison is shown the diameter distribution of cells of another tumor (EO60), a mammary adenocarcinoma that is transplantable in Little's C57 black mice.

Fig. 3 presents the experimental data of latent period and tumor volume at sacrifice for 50 mice. The plied by the average volume of the cell, 5.5×10^{-10} cc., the tumor volume is found to be 1.1×10^{-3} cc.

Fig. 3 shows that the volume of the tumor can be given approximately as an exponential function of time. Data for 7 groups of animals of the kind shown in this figure are summarized in Table I where the essential quantity μ, the characteristic coefficient in the exponential law, is determined from the equation $V = V_0 e^{\mu t}$, where V is the final volume, V_0 the volume of the cells inoculated, e is the base of the natural logarithm, μ is the growth constant, and t is the time

from inoculation to sacrifice in days. The data groups were plotted in the same manner as Fig. 3 and μ was calculated from each group. From Table I is calculated the weighted mean value of $\mu=0.37$ per day. The constant μ determines the slope of the straight line that can be drawn through the points shown in Fig. 3.

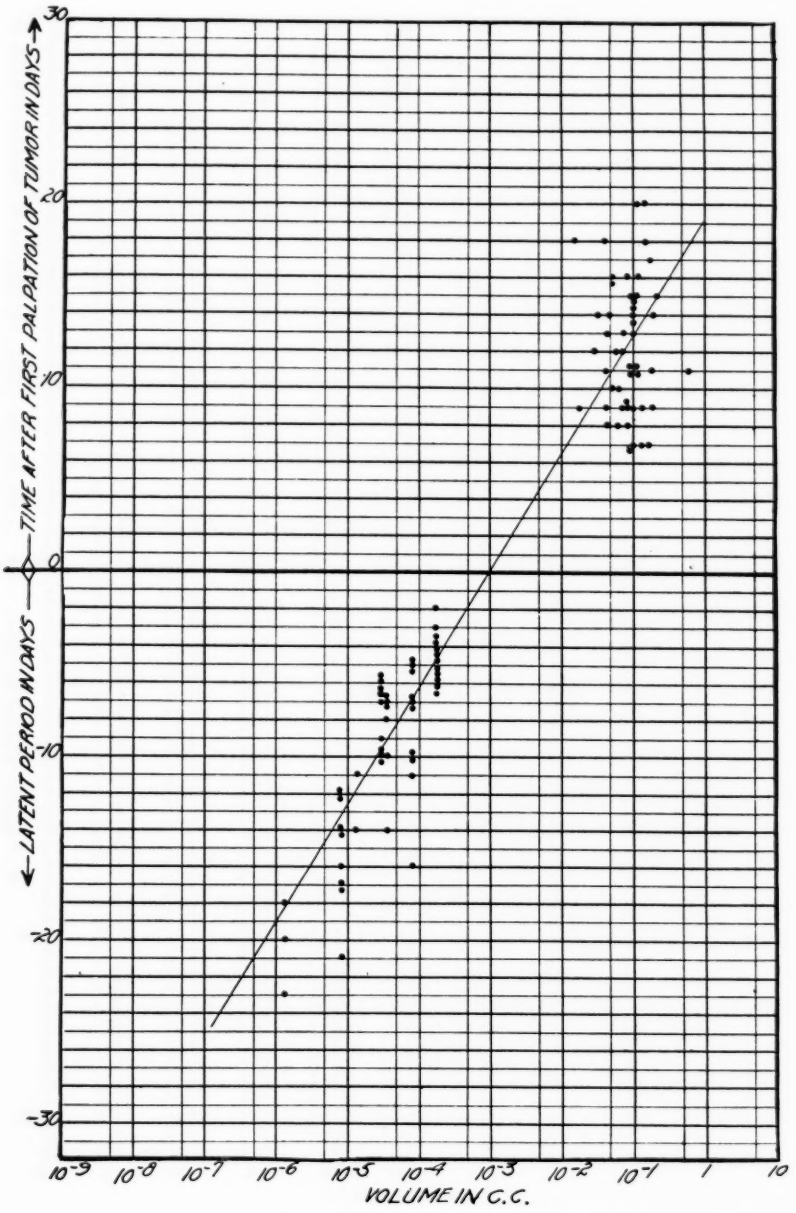

FIG. 3.—The data for one group of 50 mice show the initial volume of cells inoculated and the final volume at time of sacrifice.

TABLE I

Group No.	Number of animals	Characteristic coefficient, μ per day
1	50	0.354
2	50	0.3715
3	50	0.36
4	50	0.3365
5	50	0.3995
6	50	0.3665
7	17	0.4017
	Weighted average $\mu = 0.37$ per day	

DISCUSSION

The over-all growth curve of the tumor may be drawn from the following data: (a) the intercept at $t=0$ in Fig. 1, which gives the total number of viable cells as 2×10^6, equivalent to a volume of 1.1×10^{-3} cc. as calculated above; (b) the slope of the line shown in the semi-log plot of Fig. 3, which is found to be $\mu=0.37$ per day from the data on 317 animals summarized in Table I; and (c) the volume of the single average cell determined from the diameters shown in Fig. 2. Fig. 4 illustrates the final growth curve. The horizontal line *SS'* at the point corresponding to the

volume of a single cell gives the spread in cell volumes, which includes 99 per cent of all cells measured in Fig. 2. The horizontal line PP' at the point for $t=0$ is the spread in palpable tumor volumes defined by the dotted lines in Fig. 1.

Fig. 1 indicates that there is a correlation between inoculum size and latent period, and that the latent period is predictable once this curve has been established for the Marsh-albino mice. It should be pointed out that this strain is highly susceptible to spontaneous mammary cancer (6), having a rate of 77 per cent. Experiments with the EO60 tumor in Little's C57 black mice, which have a low susceptibility to spontaneous mammary cancer of 10 per cent (5), showed no correlation between inoculum size and latent period. Tumor takes occurred, but the nodules grew erratically and even regressed. All experiments

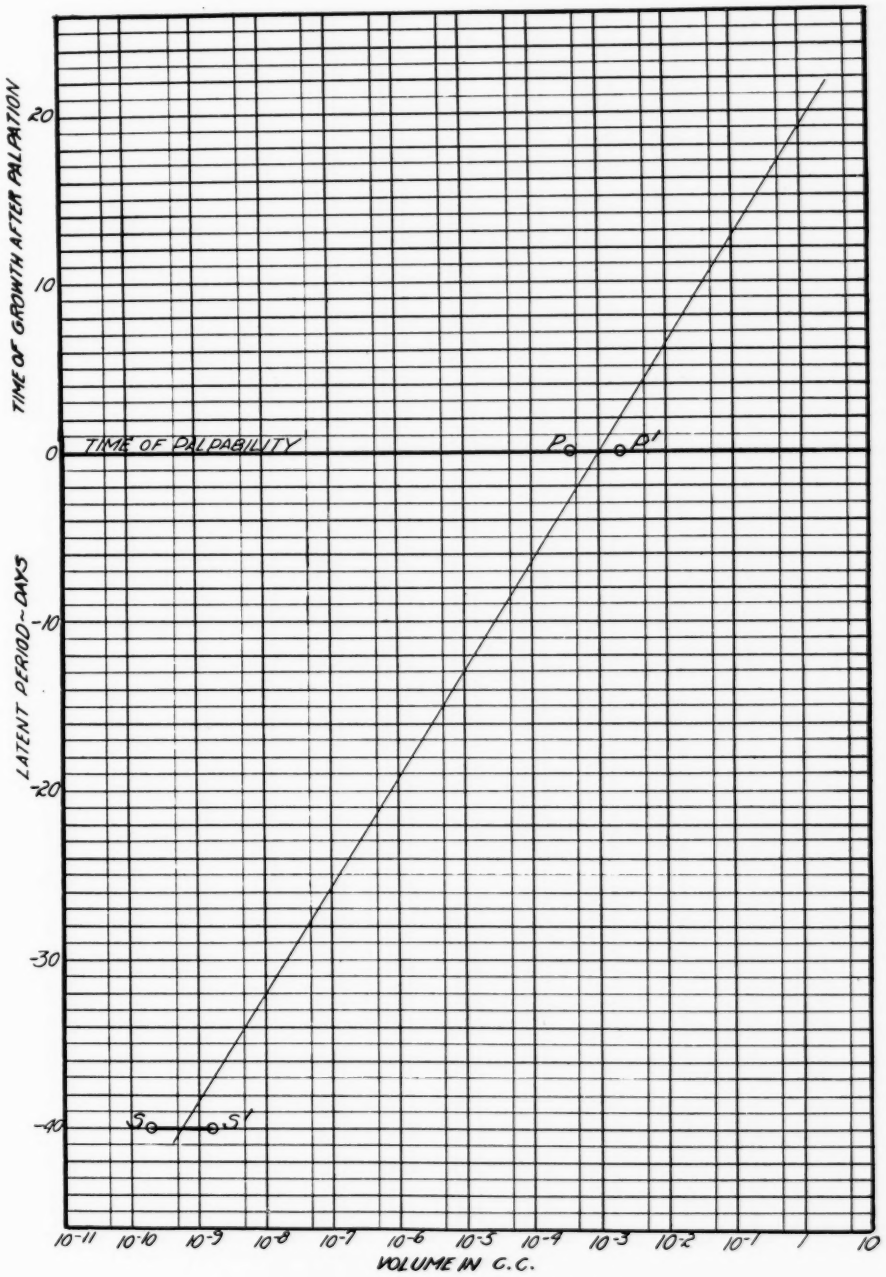

FIG. 4.—Final growth curve for Marsh-Simpson tumor.

If it be assumed in a first approximation that the cells divide by binary fission the coefficient $\mu=0.37$ per day leads to an intermitotic period of about 46 hours. If the cells divide into 3 or even 4 daughter cells the intermitotic period will be longer. Experiments are under way to determine the portion of cells that go into binary, ternary, or higher orders of fission.

in both strains were carried out with mice whose average age was 1½ months, a time well ahead of the incidence of spontaneous mammary tumors in either strain.

SUMMARY

1. A quantitative measure of the Marsh-Simpson tumor in Marsh-albino mice was carried out to establish the relation between latent period and inoculum size.

2. The growth curve is established for this tumor, beginning with a single cell.

3. The volume growth of the tumor in the mice employed is exponential with time, $V = V_0 e^{\mu t}$ where the characteristic constant $\mu = 0.37$ per day.

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Cerebral Tumor in a Dog Resembling Human Medulloblastoma

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INTRODUCTION

It is impossible at this time to attempt any satisfactory classification of spontaneous brain tumors in animals, particularly in dogs, because of the lack of sufficient pathological material for careful histological investigation. The authors, in a previous communication (3), have emphasized that the paucity of cases reported in the literature does not necessarily mean that such tumors are extremely rare. Greater frequency of postmortem examinations might reveal a much higher incidence of brain tumors in animals than is believed to exist. Such a probability is borne out by the experience of Milks and Olafson (7), who encountered brain tumors in as many as 4 per cent of their autopsies on dogs. This is a surprisingly high figure, since in man brain tumors are found in but about 1 per cent of all postmortem examinations, according to Ewing (4). It is desirable, therefore, that all brain tumors observed in animals be reported with complete gross and microscopic findings. A comparative pathological study of intracranial neoplasms should be an invaluable aid toward a better understanding of such tumors in human beings and progress in classification.

CLINICAL OBSERVATIONS

The subject, a pedigreed 3 year old male English bulldog, showed for a period of 3 months symptoms suggestive of a brain disturbance, possibly a tumor. The initial symptoms were profuse sweating, nervousness, panting, and heavy breathing during rest. Beginning with the 2nd month, there were periodic attacks of tetanic muscular spasms, during which the dog assumed a rigid standing position and refused to lie down. Any attempt to move the animal during the attacks caused him to howl as if in pain. Each of these seizures lasted for about 20 minutes. On several occasions the animal was hospitalized; the attacks were controlled by the use of sedatives. Throughout the illness the dog ate and drank well and at no time manifested any elevation of temperature. However, the animal gradually became weaker, vomited occasionally, and, during the last week or so of life, had several convulsions. Howling spells became frequent and uncontrollable. The dog was sacrificed and submitted to postmortem examination.

Gross Autopsy Findings

Postmortem investigation revealed no pathological alterations in the viscera. The brain was dissected after fixation in 10 per cent formalin solution. No lesions were found on superficial examination. A frontal section, made at the level of the anterior commissure, revealed a large, funnel-shaped tumor, $3 \times 2.5 \times 2$ cm. in dimensions, which filled most of the lateral ventricles, particularly the left (Fig. 1). The tumor appeared to arise from the wall of the left lateral ventricle in the region of the collateral trigone; from this site it extended by a broad, poorly demarcated pedicle into the ventricular cavity. Its posterior surface was corrugated, somewhat resembling cerebral convolutions. On section, the bulk of the growth was found to be grayish white, with scattered reddish or brownish areas toward the periphery. The consistency was rather firm. Anteriorly there was no line of demarcation between the tumor and the adjacent white matter of the left frontal lobe. The anterior half of the basal ganglia was replaced by neoplastic tissue. The lateral ventricles, especially posterior to the tumor, were extremely dilated. The septum pellucidum and the fornix had disappeared; the ventricles were in open communication. The corpus callosum was very atrophic.

Microscopic Findings

In addition to the routine hematoxylin-eosin and the Nissl technics, methods for myelin (Spielmeyer), neurofibrils (Bielschowsky), astrocytes (Cajal), oligodendroglia (Penfield), microglia (Hortega), glia fibers (Holzer), and mesenchymal fibers (Perdrau) were employed.

The bulk of the tumor was composed of large compact masses of cells (Fig. 2). Cell boundaries were

indiscernible. The cytoplasmic ground substance was amorphous or slightly fibrillary and faintly acidophilic. The nuclei were round or occasionally oval, vesicular, sharply outlined, and rather dark. They contained a delicate network of chromatin, with numerous granules and sometimes prominent nucleoli. Mitotic figures were frequent (Fig. 3). The arrangement of cells in rows, rosettes, or other configurations commonly observed in various forms of gliomas was absent. The tissue of the compact areas contained few blood vessels. In other regions the neoplastic cells large numbers of tumor cells infiltrated the adjacent white matter (Fig. 5). No additional characteristics, such as fibrils or other specific structures present in various forms of tumors of the nervous system, were demonstrable by any of the special staining methods.

DISCUSSION

The tumor is definitely anaplastic, somewhat resembling a round cell sarcoma, and lacks the characteristics of the more mature neoplasms of the central nervous system. It has certain similarities to ret-

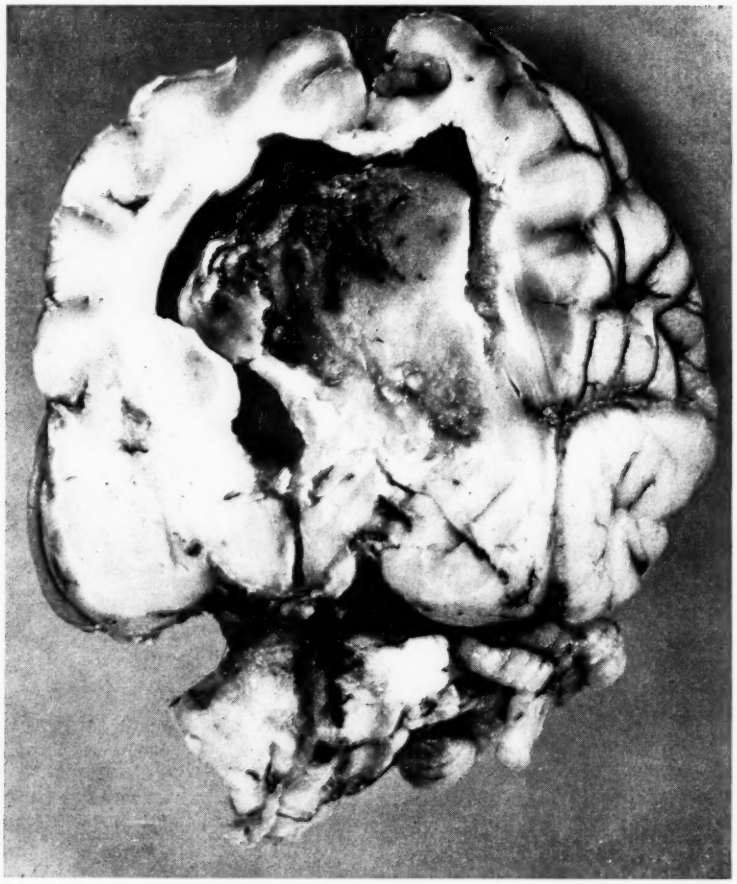

FIG. 1.—Section through cerebrum showing anterior view of tumor growing into lateral ventricles. Mag. × 1½.

were more loosely grouped but had the same individual characteristics. The tissue was richly vascularized and contained several hemorrhages. Many small vessels showed proliferation of the endothelial cells with narrowing of the lumina. At the periphery of the tumor such vessels in cross section, under low magnification, somewhat resembled renal glomeruli (Fig. 4). Thick and irregularly outlined hyaline zones surrounded some of the larger vessels. A few deposits of hemosiderin were scattered in this vicinity. Occasional small foci of necrosis were present in the peripheral zones. Although the neoplasm microscopically was fairly clearly differentiated from the brain tissue, inoblastoma, sympathicoblastoma, and especially to medulloblastoma. The relationships of these groups has been emphasized by Bailey (1). Many features of the tumor in question, especially those relative to the nuclear structure, are mentioned in the recent histologic description of the medulloblastoma by Zülch (12). The rhythmic arrangements occasionally seen in this type are absent. In differential diagnosis the oligodendroglioma and ependymoma are to be considered.

Although on superficial examination the growth may slightly resemble an oligodendroglioma, it lacks the higher degree of differentiation manifested by the presence of short argentophil processes, demonstrable

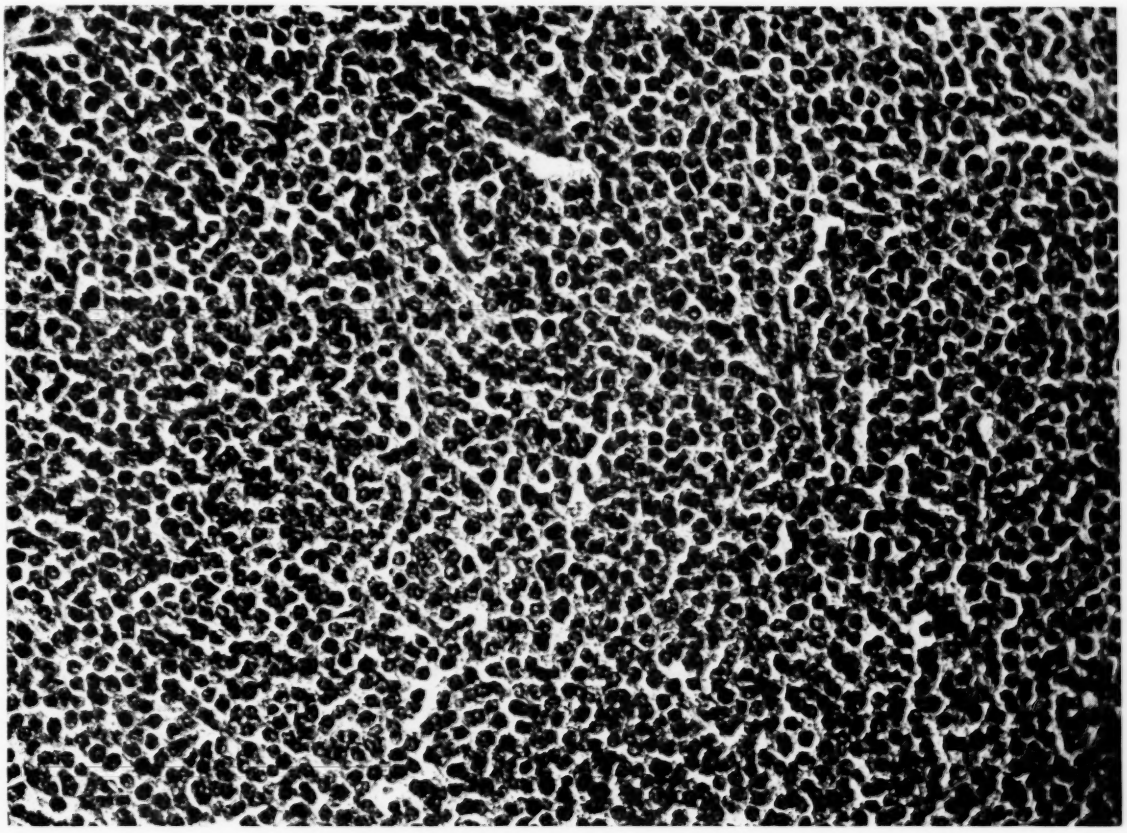

FIG. 2.—Section from solid portion of neoplasm showing compact masses of cells with roundish nuclei. Mag. × 240.

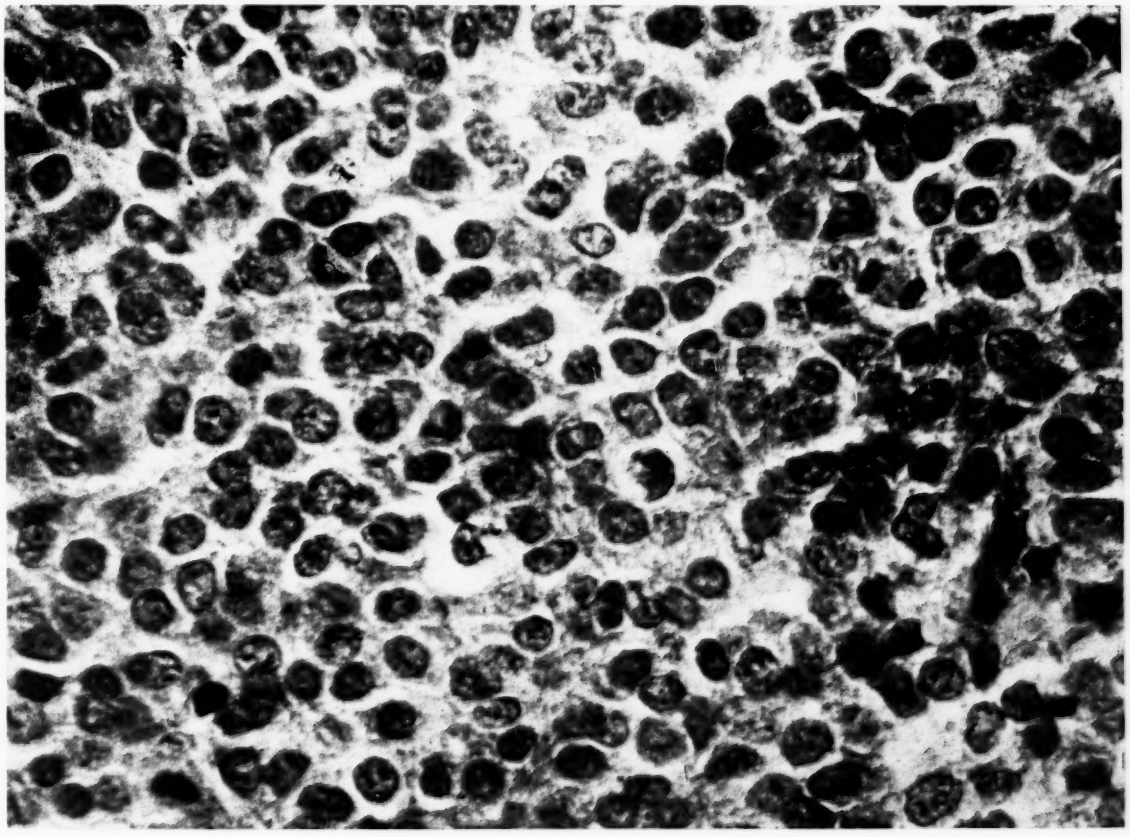

FIG. 3.—Same as Fig. 2, higher magnification, showing mitosis. Mag. × 750.

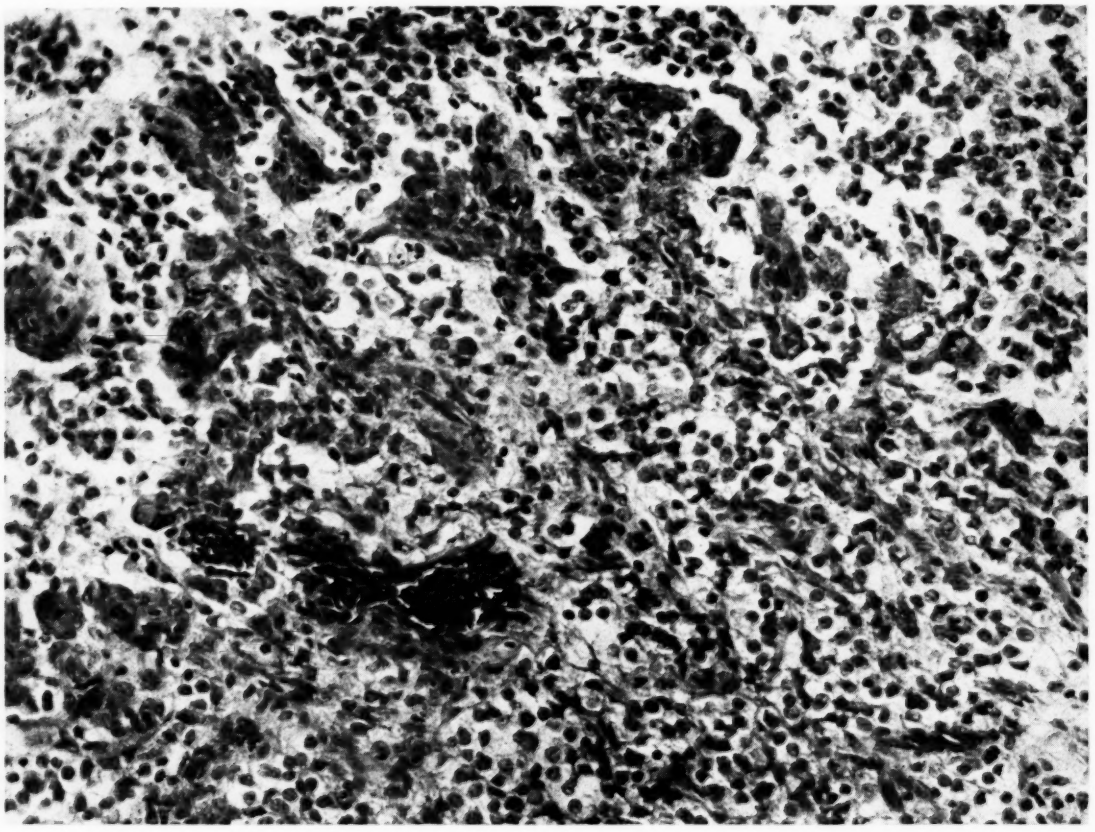

FIG. 4.—Periphery of tumor with loosely grouped cells and vascular proliferation. Mag. × 240.

FIG. 5.—Border of neoplasm showing infiltration of subcortical white matter by tumor cells. Mag. × 175.

by the Penfield method and observed by us in another tumor in the brain of a dog (3). In certain cellular ependymomas in man, to which this neoplasm in the dog may at first glance bear some similarity, the nuclei are more homogeneous, mitotic figures are scarce, and the cells do not infiltrate the adjacent brain tissue. However, "transitional" forms may possibly occur, according to Zülch (12). The medulloblastoma in man may, in exceptional cases, be found in the cerebrum (Honeyman, 5). This possibility, however, is rejected by some authors (Kershman, 6). The cerebellum is the center of predilection. In dogs, a questionable medulloblastoma, described by Batten (2), and a relatively typical medulloblastoma, reported by Milks and Olafson (7), originated in the cerebellum. However, Peers (9) found nine cerebral medulloblastomas among fifteen gliomas which he had succeeded in producing experimentally in mice by means of methylcholanthrene. In similar experiments in mice, Zimmerman and Arnold (11) reported one cerebral and three cerebellar medulloblastomas.

The point of origin of the neoplasm under discussion was probably in the periventricular layers of immature cells. Large groups of such cells, considered to be precursors of nervous and glial elements, are a normal finding in the tissue around the lateral ventricles in late fetal and early postnatal life. The development, morphology, classification, and significance of these cells have been discussed by many authors, among them Kershman (6) and Tuthill (10). That they may become the site of origin of brain tumors in man was considered a possibility by Ostertag (8). The elements of the tumor we have just described have a close resemblance to those cell accumulations.

SUMMARY

A large spontaneous cerebral tumor in a dog has been described. It obliterated most of the lateral ventricles. Histologically, the neoplasm resembled human medulloblastoma. Its probable origin was in the periventricular layers of immature cells.

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Studies on the Morphology of the Peripheral Blood of Rats*

I. Normal Rats

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Preliminary to a study of the effects of various carcinogenic agents and the growth of induced and transplanted tumors on the morphology of the blood of rats, a study was made of the variability in morphology of the blood of normal, pregnant, and lactating rats of several inbred strains. The findings were of sufficient interest to be the subject of a separate report.

The blood of the normal rat has been the subject of numerous investigations (1-8, 11-23, 25-27, 29, 30), beginning with Malassez (21) in 1875. In most investigations, however, the number of animals used has been rather small, and there are no reliable analytical data in regard to strain, age, and sex differences.

Chisholm (5) studied the hemoglobin in 50 rats and found an average of 88 per cent. Dallwig and his collaborators (11) concluded from a series of 4 animals that the average hemoglobin was 100 per cent, while Wolferth (30), using 45 rats, found it to vary from 79 to 102 per cent. Jolly (14) reported the hemoglobin slightly lower in the female than in the male. A comparison of results is impossible, however, since in no case is the hemoglobin expressed in grams and the fundamental standards may well be different.

In studying the red cell count, Jolly (14) found that the female had 0.5 to 1.0 million fewer red cells per cu. mm. than the male. Dallwig's (11) 4 animals showed counts of 7.4 to 8.5 million per cu. mm. Bedson and Zilva (1) found 7.0 to 8.5 million in 7 animals, while Cramer, Drew, and Mottram's (8) series of 10 rats varied from 9 to 10 million. Wolferth (30) studied 50 rats with red cell counts from 7.3 to 8.2 million, Klieneberger and Carl (16) 7 rats with a variation from 8.8 to 9.3 million, and Hammett and Nowrey (13) 15 animals, the counts of which varied from 7.3 to 8.3 million per cu. mm.

The literature on the white cell count is best summarized by taking the average of 134 counts done by Cramer, Drew, and Mottram (8), Goodall (12), Hammett and Nowrey (13), Linser and Helber (18), Jolly (14), Kanthack and Hardy (15), Klieneberger and Carl (16), and Taylor, Witherbee, and Murphy (29). The average total white cell count varied between 8,000 and 15,000. The average differential white blood picture, according to the same authors, was, neutrophil polymorphonuclears 27 per cent (15 to 40), lymphocytes 67 per cent (50 to 80), monocytes 5 per cent (2 to 7), eosinophils 2 per cent (0 to 4), and basophils 0.7 per cent (0 to 1.5). Rivas (25) found a somewhat greater variation in the polymorphonuclears (42 to 70 per cent) in total leucocyte counts varying from 7,000 to 16,000.

Jolly (14) found that up to 1 year of age there was an increase in the total white cell count. He also noted that pregnancy caused a slight increase in the total leucocyte count but no apparent change in the differential values. In addition, this author was of the opinion that the percentage of neutrophil polymorphonuclear leucocytes varied in different breeds of rats.

In the study to be described the rats were bled from the tail vein under light ether anesthesia. All the blood samples were taken by the same person[1] at approximately the same time of day. The hemoglobin was determined in a Sahli apparatus checked by the Van Slyke oxygen-combining method and expressed in grams per 100 cc. Blood for the red and white cell counts was taken in carefully checked pipettes, and all the counts were done by the same person using the same counting chamber at all times. It was found especially important to have the counting chamber cover slip absolutely true, as even the slightest curvature of its surface could cause considerable variation in the counts. The smears for the differential counts were all made on cover slips and stained by the combined Jenner-Giemsa method. By using these pre-

* This study was undertaken and nearly completed under the direction of Dr. F. C. Wood at the Department of Cancer Research, Columbia University.

[1] The authors acknowledge their indebtedness to Miss Sylvia Presner for careful and accurate technical assistance.

cautions it was hoped to reduce to a minimum the errors inherent in the method.

The tested rats were pedigreed and were from 9 inbred strains and a group of hybrids. All received an adequate mixed diet of bread, milk, cereal, and fresh vegetables.

Table I shows for 7 inbred strains, a group of hybrids or Silver greys, and a group of several miscellaneous strains the mean hemoglobin in grams per 100 cc., erythrocytes in millions, and leucocytes in thousands per cu. mm. with the differential values expressed in percentages. The values are given separately for males and females as well as for the sexes combined. An average value for nearly 3,000 tested rats was 14.2 gm. of hemoglobin, 8.7 million red cells, and 15.8 thousand leucocytes, 28 per cent of which were polymorphonuclear leucocytes, 64 per cent lymphocytes, 6 per cent monocytes, 2.2 per cent eosinophils, and 0.1 per cent basophils. Among the rats of different strains the average hemoglobin values varied from 12.6 ± 0.09 to 14.9 ± 0.09 gm., the erythrocytes from 8.1 ± 0.08 to 9.3 ± 0.07 million, and the leucocytes from 14.0 ± 0.10 to 26.9 ± 0.75 thousand per cu. mm. That these differences were real may be seen by comparing the hemoglobin values and the red and white cell counts for the different strains with each other statistically. Most of the differences are statistically significant, thus indicating that each strain of rats has a characteristic peripheral blood picture. The greatest difference in hemoglobin value was 1.88 ± 0.12 between the August and Copenhagen strains. In this case the difference was 15 times the probable error of the difference. The Copenhagen and Fischer strains differed most in number of red cells, the difference being 0.75 ± 0.06 million per cu. mm. and the critical ratio 12.5. The difference in number of white cells between the Copenhagen and Fischer strains was 11.1 ± 0.52 thousand per cu. mm. or 21

TABLE I: SHOWING FOR THE PERIPHERAL BLOOD OF RATS OF EACH SEX OF SEVERAL STRAINS THE MEAN HEMOGLOBIN, ERYTHROCYTES, AND LEUCOCYTES, WITH THE DIFFERENTIAL COUNT EXPRESSED IN PERCENTAGES

Group	Sex	Number of rats	Mean ± P.E. hemoglobin, gm. per 100 cc.	Mean ± P.E. R.B.C., millions per cu. mm.	Mean ± P.E. W.B.C., thousands per cu. mm.	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
August	♂	66	14.8 ± 0.12	8.8 ± 0.09	14.1 ± 0.35	29	65	4	1.4	0.03
	♀	54	14.7 ± 0.16	8.5 ± 0.10	15.2 ± 0.46	34	60	5	1.3	0.06
	Sum	120	14.7 ± 0.10	8.7 ± 0.07	14.6 ± 0.38	31	63	4	1.4	0.04
Fischer	♂	399	14.7 ± 0.04	9.2 ± 0.03	14.0 ± 0.13	25	66	7	1.9	0.01
	♀	320	14.4 ± 0.06	8.9 ± 0.04	14.0 ± 0.15	22	69	7	2.2	0.01
	Sum	719	14.6 ± 0.04	9.0 ± 0.03	14.0 ± 0.10	24	67	7	2.0	0.01
Sherman	♂	211	14.5 ± 0.06	8.8 ± 0.04	14.3 ± 0.20	28	64	6	1.9	0.00
	♀	172	14.6 ± 0.06	8.5 ± 0.04	14.5 ± 0.21	24	68	6	2.2	0.00
	Sum	383	14.6 ± 0.04	8.7 ± 0.03	14.4 ± 0.14	26	66	6	2.1	0.00
Marshall	♂	111	14.3 ± 0.09	8.9 ± 0.07	17.5 ± 0.36	27	65	6	2.0	0.01
	♀	139	14.4 ± 0.07	8.6 ± 0.06	16.8 ± 0.31	26	66	6	2.7	0.01
	Sum	250	14.3 ± 0.06	8.7 ± 0.04	17.1 ± 0.24	26	65	6	2.4	0.01
A × C	♂	114	13.9 ± 0.07	8.5 ± 0.06	17.0 ± 0.38	36	56	5	3.0	0.01
	♀	114	14.2 ± 0.10	8.4 ± 0.06	18.0 ± 0.53	33	59	5	3.4	0.01
	Sum	228	14.0 ± 0.06	8.4 ± 0.04	17.5 ± 0.33	34	57	5	3.2	0.01
Silver grey	♂	156	14.2 ± 0.06	8.6 ± 0.05	14.6 ± 0.29	29	64	5	1.8	0.03
	♀	139	13.8 ± 0.07	8.4 ± 0.06	15.5 ± 0.31	25	67	6	1.7	0.00
	Sum	295	14.0 ± 0.05	8.5 ± 0.04	15.0 ± 0.21	27	66	5	1.7	0.02
Zimmerman	♂	135	13.8 ± 0.08	8.8 ± 0.06	15.3 ± 0.30	27	65	6	1.6	0.01
	♀	152	13.2 ± 0.09	8.2 ± 1.1	15.8 ± 0.34	25	67	7	2.1	0.00
	Sum	287	13.5 ± 0.06	8.5 ± 0.04	15.5 ± 0.23	26	66	6	1.9	0.00
Copenhagen	♂	87	13.1 ± 0.08	8.5 ± 0.07	23.3 ± 0.68	39	53	6	2.0	0.00
	♀	87	12.6 ± 0.09	8.1 ± 0.08	26.9 ± 0.75	38	53	6	3.4	0.00
	Sum	174	12.8 ± 0.06	8.3 ± 0.05	25.1 ± 0.51	38	53	6	2.7	0.00
Miscellaneous	♂	87	14.9 ± 0.09	9.3 ± 0.07	14.0 ± 0.33	30	63	5	2.7	0.00
	♀	113	14.6 ± 0.10	8.9 ± 0.06	16.3 ± 0.38	30	62	5	2.6	0.00
	Sum	200	14.8 ± 0.07	9.1 ± 0.05	15.3 ± 0.26	30	62	5	2.6	0.00
Total	♂	1,366	14.3 ± 0.02	8.9 ± 0.02	15.4 ± 0.10	29	63	6	2.0	0.01
	♀	1,290	14.1 ± 0.03	8.6 ± 0.02	16.2 ± 0.12	27	65	6	2.4	0.01
	Sum	2,656	14.2 ± 0.02	8.7 ± 0.01	15.8 ± 0.08	28	64	6	2.2	0.01

times the probable error of the difference. It may be noted further that one strain, the Copenhagen, was strikingly different from all the others. It had the lowest hemoglobin value and red cell count and the highest leucocyte count with a characteristically high percentage of polymorphonuclears.

hemoglobin value was slightly lower for most of the rats under 100 days of age, but after 100 days no consistent differences were observed. The white cell counts tended to be lower in rats under 100 days of age and to increase more or less consistently with age. The most conspicuous differences, however, were

TABLE II: COMPOSITION OF THE PERIPHERAL BLOOD OF RATS OF SEVERAL STRAINS AT DIFFERENT AGE LEVELS

Age, days	Strain	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
50	Fischer	34	14.6 ± 0.16	9.4 ± 0.08	14.2 ± 0.35	19	69	11	1.6	0.00
	Marshall	19	13.7 ± 0.17	9.2 ± 0.17	14.9 ± 0.48	22	70	6	1.3	0.05
	A × C	18	13.2 ± 0.17	8.1 ± 0.10	16.8 ± 0.46	26	64	6	4.4	0.00
	Sherman	19	13.0 ± 0.15	8.4 ± 0.10	12.9 ± 0.50	14	76	8	1.4	0.00
	Silver grey	14	13.4 ± 0.21	8.3 ± 0.13	14.4 ± 0.54	14	76	8	2.1	0.07
	Total	113	13.7 ± 0.09	8.9 ± 0.06	14.3 ± 0.23	19	71	8	2.0	0.02
100	Fischer	442	14.7 ± 0.04	9.1 ± 0.03	13.9 ± 0.10	22	69	7	2.0	0.01
	August	41	14.2 ± 0.14	8.7 ± 0.09	14.1 ± 0.45	26	68	4	1.2	0.02
	Copenhagen	67	12.8 ± 0.09	8.2 ± 0.08	24.6 ± 0.90	33	58	6	3.0	0.00
	Marshall	90	13.7 ± 0.17	8.8 ± 0.07	18.0 ± 0.36	24	68	6	1.8	0.00
	Zimmerman	123	13.5 ± 0.08	8.7 ± 0.06	15.2 ± 0.28	20	72	6	1.8	0.00
	A × C	60	13.5 ± 0.08	8.4 ± 0.07	16.2 ± 0.48	27	64	5	3.5	0.00
	Sherman	57	14.3 ± 0.10	8.3 ± 0.07	14.9 ± 0.28	19	74	5	2.3	0.00
	Silver grey	114	14.1 ± 0.08	8.5 ± 0.06	15.2 ± 0.33	25	69	4	1.8	0.01
	Total	1,038	14.3 ± 0.03	8.8 ± 0.02	15.5 ± 0.12	24	68	6	2.1	0.01
200	Fischer	124	14.3 ± 0.10	8.7 ± 0.07	13.9 ± 0.29	24	68	6	1.7	0.00
	August	43	15.4 ± 0.20	8.6 ± 0.13	14.7 ± 0.42	30	65	4	1.6	0.05
	Copenhagen	53	12.9 ± 0.12	8.1 ± 0.08	25.6 ± 0.78	42	49	6	2.6	0.00
	Marshall	80	14.2 ± 0.09	8.6 ± 0.06	16.7 ± 0.40	27	64	6	2.7	0.00
	Zimmerman	77	13.2 ± 0.15	8.1 ± 0.09	16.1 ± 0.54	30	62	7	2.0	0.01
	A × C	95	14.5 ± 0.10	8.4 ± 0.06	16.8 ± 0.44	37	55	5	2.9	0.02
	Sherman	148	14.8 ± 0.06	8.8 ± 0.05	14.8 ± 0.11	24	68	5	2.4	0.00
	Silver grey	94	14.0 ± 0.08	8.4 ± 0.07	13.9 ± 0.34	30	63	5	1.5	0.02
	Total	779	14.3 ± 0.04	8.5 ± 0.02	15.9 ± 0.15	29	63	5	2.2	0.01
300	Fischer	108	14.5 ± 0.09	9.0 ± 0.07	14.7 ± 0.31	31	60	6	2.4	0.05
	August	23	14.2 ± 0.19	8.9 ± 0.16	15.9 ± 0.65	37	56	6	1.4	0.00
	Copenhagen	40	12.8 ± 0.14	8.7 ± 0.12	26.4 ± 1.0	42	49	6	2.5	0.00
	Marshall	36	14.3 ± 0.21	8.8 ± 0.14	18.7 ± 0.77	29	61	6	3.1	0.03
	Zimmerman	74	13.6 ± 0.12	8.5 ± 0.09	16.1 ± 0.47	31	60	6	2.0	0.00
	A × C	45	14.0 ± 0.12	8.8 ± 0.10	20.6 ± 1.1	40	52	5	3.3	0.00
	Sherman	112	14.5 ± 0.08	8.8 ± 0.06	14.2 ± 0.28	31	61	6	1.6	0.01
	Silver grey	51	14.0 ± 0.11	8.6 ± 0.10	17.0 ± 0.61	28	64	6	2.0	0.00
	Total	534	14.2 ± 0.04	8.8 ± 0.03	16.8 ± 0.21	32	60	6	2.2	0.01
400	Fischer	11	13.3 ± 0.37	8.7 ± 0.12	12.8 ± 1.1	32	60	6	2.9	0.00
	August	13	14.3 ± 0.15	8.6 ± 0.21	13.6 ± 1.3	42	52	5	1.2	0.15
	Copenhagen	14	13.3 ± 0.27	8.3 ± 0.17	21.7 ± 2.0	41	52	5	2.3	0.00
	Marshall	25	14.8 ± 0.16	8.2 ± 0.11	15.1 ± 0.82	34	58	5	3.0	0.00
	A × C	10	13.8 ± 0.42	8.4 ± 0.39	19.8 ± 2.0	42	53	4	1.9	0.00
	Sherman	47	14.8 ± 0.10	8.7 ± 0.10	13.9 ± 0.53	35	57	6	2.1	0.00
	Silver grey	22	13.7 ± 0.17	8.5 ± 0.11	15.5 ± 0.80	37	54	7	1.6	0.00
	Total	187	14.3 ± 0.07	8.7 ± 0.05	15.4 ± 0.34	36	56	6	2.2	0.01

In Table II are given the blood pictures of rats of several strains at different age levels. The youngest rats tested were from 50 to 100 days of age and the oldest from 400 to 600 days. Most of the latter group were between 400 and 500 days of age and therefore included very few rats as old as the average age for spontaneous tumors; *i.e.*, 20.6 ± 0.2 months (9). The

in the differential values, the percentage of polymorphonuclear leucocytes increasing with age and the percentage of lymphocytes and of monocytes being highest in the youngest age group. These findings are comparable to the age differences observed in human differential counts. Statistical comparisons of the mean values for hemoglobin and for red and white cell

counts of the youngest age group with the corresponding values of the older age groups show that with the exception of the Fischer rats the mean hemoglobin values and the white cell counts were significantly lower in rats under 100 days of age. The red cell counts were not consistently different in any age group. A possible explanation of the exception in the case of the Fischer rats was thought to be the fact that they mature earlier than the others. Comparable values might be expected in rats of this strain between 30 and 60 days of age.

Some time after this series had been completed, it was possible to obtain similar counts of embryos and young rats of two strains. The findings are summarized in Table III and include counts of nearly full-term embryos, rats the first week after birth, and rats at weaning or 28 to 40 days of age. The age differences between these groups were more apparent. Progressive increases in the hemoglobin value and in the number of red cells were observed with increase in age. The white cell counts were more variable but with the exception of the counts of Fischer embryos, which seemed disproportionally high, were significantly lower than were the counts for the youngest group shown in Table II (50 to 100 days). The differential values were characterized by relatively high percentages of monocytes. At these early ages no sex differences were observed, but significant strain differences in hemoglobin and red cell counts were apparent. Rats of the Fischer strain, which had the highest hemoglobin and red cell counts among the youngest rats considered in Table II, apparently attained these high values between the 1st and 5th weeks of age.

Differences between the sexes were not very consistent. Statistical comparisons of the separate values from Table I show that for the total of the rats of all strains the males differed from the females by 0.23 ± 0.04 gm. of hemoglobin per 100 cc. and by 0.32 ± 0.03 million red blood cells per cu. mm. The females had a significantly higher and more variable white cell count, the difference being 0.82 ± 0.16 thousand per cu. mm. In some of the strains there were notable exceptions. The Marshall males tested had a slightly higher white cell count than the females. Fischer males and females showed no difference in white cell count for all ages combined or for the largest group of 100 to 200 days. The differences in white cell count were insignificant for all rats under 200 and over 400 days of age. The greatest difference in white cell count was 3.65 ± 1.0 between males and females of the Copenhagen strain, the strain with the highest total white cell count. The males had a consistently higher red cell count than the females, and most of the differences were significant, an exception being found in rats of the A×C strain, which had almost no difference. Females of the Sherman, Marshall, and A×C strains had higher hemoglobin values than the males, but the differences were insignificant. The greatest difference in hemoglobin was 0.68 ± 0.12 gm. per 100 cc. between males and females of the Zimmerman strain.

Before attempting an interpretation of the changes

TABLE III: COMPOSITION OF THE PERIPHERAL BLOOD OF EMBRYOS AND YOUNG RATS OF THE FISCHER AND A × C STRAINS

Numbers inclosed in parentheses are the percentages of immature polymorphonuclear leucocytes

Age	Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Embryo	A × C	68	9.8 ± 0.09	1.3 ± 0.05	6.5 ± 0.22	20(7)	46	27	0.04	0.01
	Fischer	106	8.8 ± 0.06	1.3 ± 0.03	15.5 ± 0.51	23(7)	43	27	0.11	0.04
	Total	174	9.2 ± 0.06	1.3 ± 0.03	12.0 ± 0.39	22(7)	44	27	0.09	0.03
1 to 8 days	A × C ♂	34	10.1 ± 0.14	2.4 ± 0.13	5.4 ± 0.27	25	54	21	0.00	0.00
	A × C ♀	32	10.7 ± 0.18	2.4 ± 0.10	5.5 ± 0.46	26	54	20	0.00	0.03
	Sum	66	10.4 ± 0.12	2.4 ± 0.08	5.4 ± 0.16	26	54	20	0.00	0.02
	Fischer ♂	49	9.1 ± 0.09	1.6 ± 0.05	5.8 ± 0.32	26	48	25	0.02	0.00
	Fischer ♀	48	9.0 ± 0.08	1.7 ± 0.09	6.1 ± 0.33	24(4)	45	27	0.00	0.02
	Sum	97	9.0 ± 0.06	1.7 ± 0.05	6.0 ± 0.23	25(2)	47	26	0.01	0.01
	Total	163	9.6 ± 0.07	2.0 ± 0.05	5.8 ± 0.15	25(2)	49	23	0.01	0.01
28 to 40 days	A × C ♂	22	11.6 ± 0.10	5.1 ± 0.08	6.6 ± 0.36	13	67	20	0.00	0.00
	A × C ♀	30	11.6 ± 0.11	5.1 ± 0.08	6.7 ± 0.25	13(3)	63	21	0.00	0.00
	Sum	52	11.6 ± 0.08	5.1 ± 0.06	6.6 ± 0.21	13(2)	65	20	0.00	0.00
	Fischer ♂	27	12.5 ± 0.21	5.4 ± 0.13	7.2 ± 0.32	11	65	24	0.00	0.00
	Fischer ♀	39	12.4 ± 0.14	5.6 ± 0.10	7.0 ± 0.20	14	62	23	0.18	0.00
	Sum	66	12.4 ± 0.12	5.5 ± 0.08	7.1 ± 0.18	13	63	24	0.11	0.00
	Total	118	12.1 ± 0.08	5.3 ± 0.05	6.9 ± 0.14	13(1)	64	22	0.06	0.00

observed in the peripheral blood pictures of rats bearing transplanted and induced tumors, it seemed desirable to record the effects produced by pregnancy and lactation. Accordingly tests were made on 140 pregnant and 214 lactating females. The results are recorded in Tables IV and V. Separate tabulations are given for females whose subsequent records showed that they bore less than five fetuses or more than five fetuses and for females that were suckling less than five or more than five young. These tables show that in neither case was the number of fetuses or young a factor in the changes observed. A glance at the

TABLE IV: COMPOSITION OF THE PERIPHERAL BLOOD OF PREGNANT FEMALE RATS WITH 1 TO 5 AND WITH 6 OR MORE FETUSES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Fischer									
1 to 5 fetuses	15	12.4 ± 0.22	8.2 ± 0.17	12.7 ± 0.40	28	64	6	1.7	0.00
6 + fetuses	25	12.9 ± 0.14	8.1 ± 0.15	14.0 ± 0.57	27	63	7	2.8	0.00
Sum	40	12.7 ± 0.12	8.1 ± 0.11	13.6 ± 0.39	28	63	6	2.4	0.00
Zimmerman									
1 to 5 fetuses	7	12.5 ± 0.27	7.1 ± 0.23	14.1 ± 0.60	26	66	5	3.3	0.00
6 + fetuses	6	12.3 ± 0.19	7.5 ± 0.17	11.7 ± 0.88	21	69	8	1.8	0.00
Sum	14	12.4 ± 0.16	7.2 ± 0.15	12.9 ± 0.54	24	67	7	2.5	0.00
A × C									
1 to 5 fetuses	27	13.2 ± 0.18	8.5 ± 0.13	18.0 ± 0.72	30	61	5	3.7	0.04
6 + fetuses	13	13.1 ± 0.25	8.3 ± 0.19	17.5 ± 0.62	24	64	7	4.2	0.00
Sum	42	13.2 ± 0.14	8.4 ± 0.11	18.0 ± 0.57	28	62	6	3.8	0.02
Miscellaneous									
1 to 5 fetuses	20	13.8 ± 0.19	8.8 ± 0.12	20.7 ± 1.5	32	57	8	3.2	0.00
6 + fetuses	22	13.1 ± 0.23	8.3 ± 0.19	17.4 ± 0.75	29	61	7	3.0	0.00
Total									
1 to 5 fetuses	69	13.1 ± 0.11	8.4 ± 0.08	17.2 ± 0.58	30	61	7	3.1	0.01
6 + fetuses	66	13.0 ± 0.11	8.2 ± 0.10	15.6 ± 0.40	27	63	7	3.0	0.00
Sum	140	13.0 ± 0.08	8.3 ± 0.06	16.5 ± 0.36	28	62	7	3.0	0.01

TABLE V: COMPOSITION OF THE PERIPHERAL BLOOD OF LACTATING FEMALES SUCKLING 1 TO 5 AND SUCKLING 6 OR MORE YOUNG

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Fischer									
1 to 5 young	20	12.4 ± 0.32	8.4 ± 0.18	15.7 ± 0.56	26	64	7	2.7	0.00
6 + young	35	13.2 ± 0.18	8.2 ± 0.13	14.9 ± 0.52	29	62	7	2.2	0.03
Sum	55	12.9 ± 0.17	8.3 ± 0.11	15.2 ± 0.39	28	63	7	2.4	0.02
Zimmerman									
1 to 5 young	19	12.3 ± 0.17	7.2 ± 0.22	16.4 ± 0.88	33	55	10	2.2	0.00
6 + young	20	12.2 ± 0.40	7.2 ± 0.22	13.9 ± 0.89	30	62	6	1.6	0.00
Sum	39	12.2 ± 0.22	7.2 ± 0.16	15.1 ± 0.64	32	58	8	1.9	0.00
A × C									
1 to 5 young	20	13.2 ± 0.21	8.6 ± 0.16	19.6 ± 1.4	32	60	5	3.2	0.00
6 + young	28	13.2 ± 0.15	8.8 ± 0.15	15.2 ± 0.52	30	60	7	3.0	0.00
Sum	50	13.1 ± 0.14	8.6 ± 0.12	17.4 ± 0.70	31	60	6	3.0	0.00
Miscellaneous									
1 to 5 young	30	13.4 ± 0.19	8.4 ± 0.13	18.4 ± 0.78	32	60	6	1.6	0.00
6 + young	38	12.7 ± 0.17	8.2 ± 0.12	16.9 ± 0.62	32	60	6	1.3	0.03
Total									
1 to 5 young	89	12.9 ± 0.12	8.2 ± 0.09	17.6 ± 0.48	31	60	7	2.3	0.00
6 + young	121	12.9 ± 0.11	8.2 ± 0.08	15.5 ± 0.32	30	61	7	2.0	0.02
Sum	214	12.9 ± 0.08	8.2 ± 0.06	16.5 ± 0.28	31	60	7	2.1	0.01

tables reveals further that the differential values were essentially the same as for the nonpregnant females shown in Table I. The hemoglobin values, however, were considerably lower in both pregnant and lactating females.

In Table VI are tabulated the differences in mean hemoglobin value and in red and white cell counts between normal females and pregnant and lactating females of the same strain. With one exception the drop in red cell count, but the differences were greater in the Zimmerman strain where both small numbers and greater variability, however, made the differences statistically insignificant. In the A × C strain the small insignificant differences were in the opposite direction. The total white cell counts were very erratic, being increased in some strains and decreased in others.

One further variable was the time element (Table VII, where the pregnant and lactating females are

TABLE VI: DIFFERENCES IN MEAN HEMOGLOBIN VALUES AND IN RED AND WHITE CELL COUNTS OF THE PERIPHERAL BLOOD BETWEEN NORMAL FEMALES AND PREGNANT AND LACTATING FEMALES OF THE SAME STRAIN

Strain	Pregnant females		Lactating females	
	Difference ± P.E.	Difference / P.E. difference	Difference ± P.E.	Difference / P.E. difference
MEAN HEMOGLOBIN VALUE				
August			0.74 ± 0.37	2.0
Copenhagen	— 0.34 ± 0.24	1.4	— 0.30 ± 0.24	1.2
Fischer	1.70 ± 0.13	13.2	1.51 ± 0.18	8.4
Marshall	1.17 ± 0.41	2.9	1.71 ± 0.34	5.0
Zimmerman	0.73 ± 0.18	4.1	0.94 ± 0.24	3.9
A × C	0.92 ± 0.17	5.4	1.08 ± 0.17	6.4
Silver grey	0.84 ± 0.49	1.7	0.90 ± 0.29	3.1
Miscellaneous	1.13 ± 0.28	4.0	1.30 ± 0.28	4.6
Total	1.07 ± 0.09	11.9	1.25 ± 0.09	13.9
MEAN RED CELL COUNT				
August			— 0.74 ± 0.33	2.2
Copenhagen	— 0.44 ± 0.19	2.3	0.22 ± 0.20	1.1
Fischer	0.75 ± 0.12	6.2	0.59 ± 0.12	4.9
Marshall	0.25 ± 0.37	0.7	0.40 ± 0.24	1.7
Zimmerman	1.03 ± 1.1	0.9	0.99 ± 1.1	0.9
A × C	— 0.03 ± 0.13	0.2	— 0.19 ± 0.13	1.5
Silver grey	0.29 ± 0.30	1.0	0.38 ± 0.40	1.0
Miscellaneous	0.27 ± 0.20	1.4	0.21 ± 0.19	1.1
Total	0.31 ± 0.06	4.9	0.38 ± 0.06	6.0
MEAN WHITE CELL COUNT				
August			— 1.49 ± 1.1	1.4
Copenhagen	2.3 ± 2.4	1.0	6.50 ± 1.3	5.1
Fischer	0.4 ± 0.43	1.0	— 1.23 ± 0.42	3.0
Marshall	— 1.3 ± 1.8	0.7	— 2.35 ± 1.5	1.6
Zimmerman	2.9 ± 0.64	4.6	0.65 ± 0.73	0.9
A × C	0.09 ± 0.78	0.0	0.57 ± 0.88	0.7
Silver grey	— 3.73 ± 1.5	2.5	0.02 ± 1.0	0.0
Miscellaneous	— 0.11 ± 0.90	0.1	— 0.46 ± 0.80	0.6
Total	— 0.28 ± 0.38	0.8	— 0.30 ± 0.31	1.0

mean hemoglobin value was lower in the pregnant and lactating females than in the normal females of the same strain, and in most instances the differences were statistically significant. The maximum difference, 1.71 ± 0.34 gm. per 100 cc., was observed in the Marshall females that were suckling young. The exception mentioned above was found in females of the Copenhagen strain, which normally have the lowest hemoglobin value observed in this series. The differences in red and white cell counts were less consistent. Fischer females had the most significant

grouped according to the weekly periods in pregnancy and suckling). In each case females of the Fischer and A × C strains were tabulated separately. The differential values were not much altered except for a slight increase in neutrophil polymorphonuclears during the 2nd week after parturition. Statistical comparisons of mean hemoglobin values between normal females and females in each weekly period from fertilization to weaning show that from the 8th day of pregnancy until the beginning of the 4th week of lactation the hemoglobin was significantly lower

than in the normal females. A maximum average decrease of more than 2 gm. per 100 cc. was observed during the first week after birth; this value gradually returned to normal during the next 2 weeks although the females were suckling young. A similar cyclic decrease was noted in the red cell counts, while changes in the white cell counts were erratic and for the most part insignificant. Figs. 1 and 2 are graphic representations of the cyclical changes in the hemoglobin values and in the red cell counts during pregnancy and lactation.

TABLE VII: COMPOSITION OF THE PERIPHERAL BLOOD OF RATS AT WEEKLY INTERVALS DURING PREGNANCY AND AFTER PARTURITION

Group	Strain	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
PREGNANT										
1 to 7 days	Fischer	8	13.9 ± 0.26	9.1 ± 0.28	15.2 ± 0.97	31	60	6	3.2	0.00
	A × C	16	13.6 ± 0.17	8.9 ± 0.13	17.5 ± 0.68	25	63	7	5.2	0.00
	Total	41	13.8 ± 0.11	9.0 ± 0.09	19.0 ± 0.84	27	62	7	4.1	0.00
8 to 14 days	Fischer	15	12.9 ± 0.18	8.5 ± 0.14	13.3 ± 0.55	29	63	6	2.1	0.00
	A × C	13	12.9 ± 0.24	8.0 ± 0.16	19.3 ± 1.2	32	59	5	4.0	0.00
	Total	45	13.1 ± 0.13	8.2 ± 0.11	15.9 ± 0.52	30	61	6	3.0	0.00
15 to 21 days	Fischer	17	12.0 ± 0.11	7.4 ± 0.09	13.0 ± 0.60	25	66	7	2.2	0.00
	A × C	13	13.2 ± 0.31	8.3 ± 0.21	17.3 ± 1.1	29	64	5	1.8	0.08
	Total	54	12.4 ± 0.12	7.7 ± 0.09	15.1 ± 0.45	29	62	7	2.3	0.02
AFTER PARTURITION										
1st week	Fischer	13	12.0 ± 0.39	8.1 ± 0.27	15.0 ± 0.66	23	68	7	1.9	0.00
	A × C	13	11.8 ± 0.33	7.7 ± 0.26	20.8 ± 1.5	33	57	6	3.6	0.00
	Total	61	12.1 ± 0.14	7.6 ± 0.12	17.0 ± 0.49	29	63	6	1.9	0.00
2nd week	Fischer	18	12.6 ± 0.21	8.1 ± 0.18	15.1 ± 0.74	32	59	7	2.1	0.00
	A × C	10	12.9 ± 0.14	8.4 ± 0.11	16.2 ± 1.5	33	58	6	3.9	0.00
	Total	56	12.9 ± 0.14	8.1 ± 0.10	15.1 ± 0.53	33	58	7	2.3	0.00
3rd week	Fischer	6	11.5 ± 0.45	8.0 ± 0.27	15.3 ± 0.81	25	67	6	2.5	0.00
	A × C	10	13.3 ± 0.25	8.7 ± 0.26	14.2 ± 0.84	29	63	6	1.8	0.00
	Total	30	12.4 ± 0.28	8.0 ± 0.19	16.5 ± 0.78	31	61	6	1.9	0.00
4th week	Fischer	18	14.3 ± 0.19	8.8 ± 0.15	15.4 ± 0.77	29	60	8	3.1	0.06
	A × C	17	14.0 ± 0.16	9.4 ± 0.12	17.5 ± 1.2	29	62	6	2.6	0.00
	Total	67	13.8 ± 0.11	8.9 ± 0.09	17.3 ± 0.51	31	60	7	2.2	0.03

DISCUSSION

Aside from recording the limits of variation to be expected in normal rats of several closely inbred strains and the changes encountered during pregnancy and lactation, two problems of general interest were suggested by the present study. The first was the subject of a preliminary report (24) and is presented graphically in Fig. 3, where the total leucocyte count and the percentage of neutrophil polymorphonuclears are plotted for each of six strains. Superimposed on these values are the mean life span values in months, which had been determined (10) for normal rats of these strains. A striking parallelism between the relative percentage values of neutrophil polymorphonuclears observed and the expected average life span is apparent. The exception in the case of A × C rats is probably due to the fact that the average life span value was obtained for rats of the first 7 brother by sister generations of this strain, which was of hybrid origin. The blood tests were obtained on rats that had been inbred at least another 10 generations and would presumably have a somewhat lower average life span. The parallelism mentioned above suggests that the association of a relatively high white cell count and a relatively high percentage of neutrophil polymorphonuclear leucocytes with a long average life span may be more than accidental.

A second problem of interest centers about the fact that rats of the Copenhagen strain had a significantly lower hemoglobin value than any of the others tested. These rats, as noted in a previous report (9), had a high spontaneous tumor history. A nearly completed study of the spontaneous tumor records of rats of this strain and those of the Fischer strain shows notable differences at all ages and for nearly all kinds of tumors. The parallelism here may be merely coincidental, but a recent report of Strong and Francis (28) of lower hemoglobin values for strain A mice, which

have a high incidence of spontaneous mammary cancer, lends it additional weight.

2. Rats under 100 days of age were found to have lower hemoglobin values and red and white cell counts

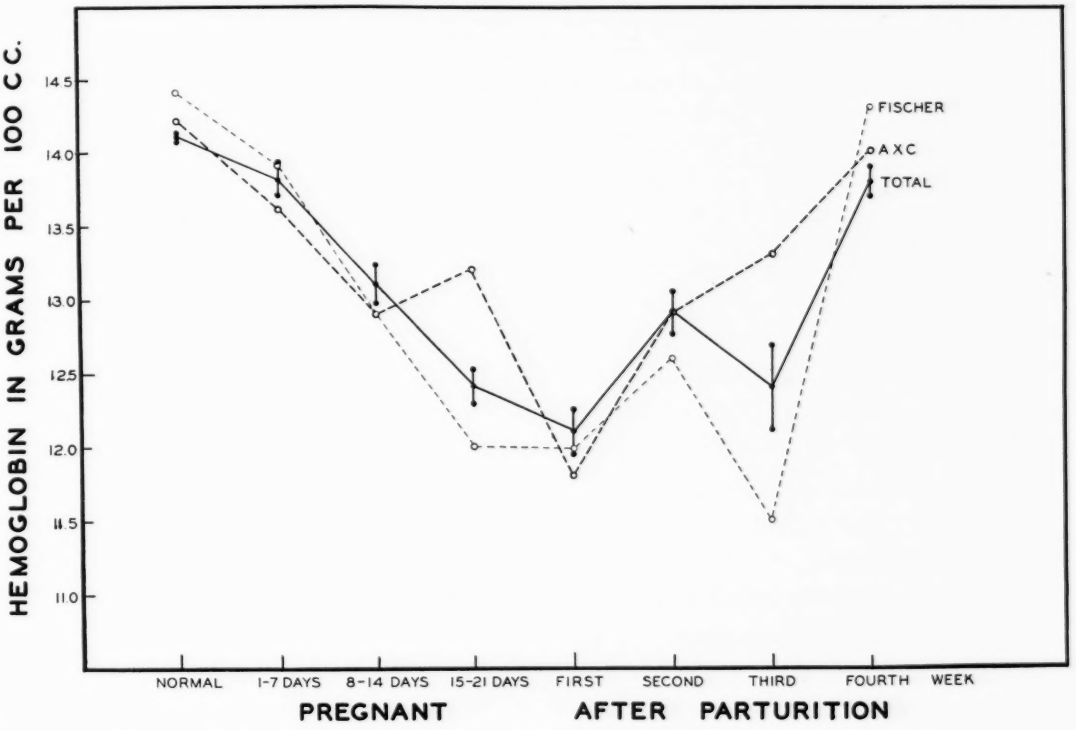

FIG. 1.—Cyclic changes in mean hemoglobin values during pregnancy and lactation.

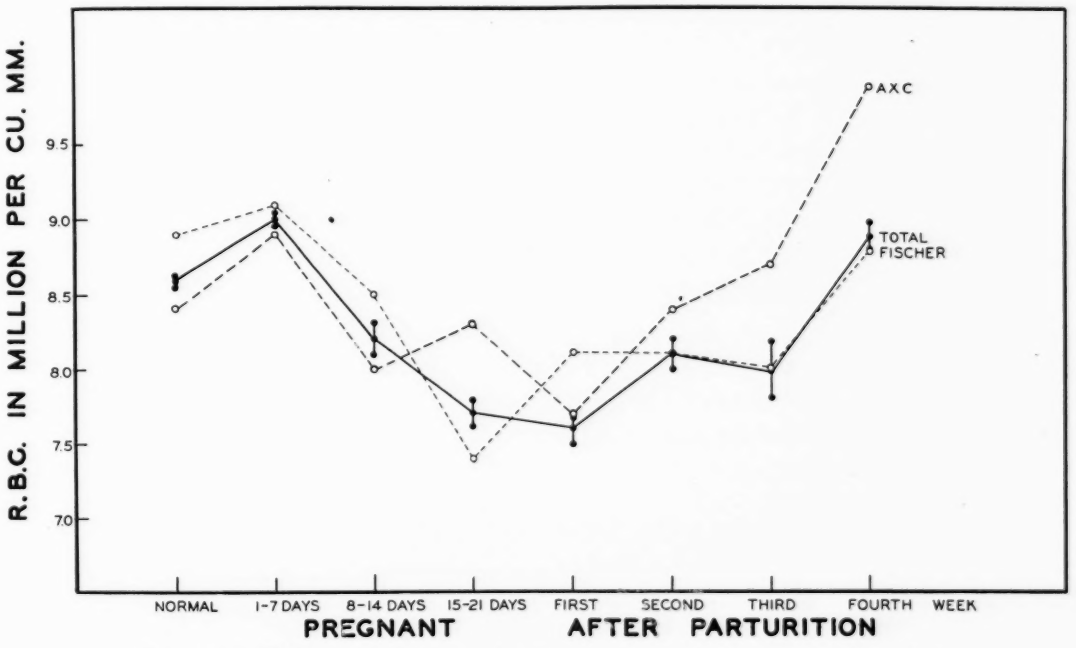

FIG. 2.—Cyclic changes in mean red cell counts during pregnancy and lactation.

SUMMARY

1. Significant differences were observed in the mean hemoglobin values and in the red and white cell counts of the peripheral blood of rats of several inbred strains. with a lower percentage of polymorphonuclear leucocytes and higher percentages of lymphocytes and monocytes than older rats, but after this period little change was observed with increase in age up to 500 days.

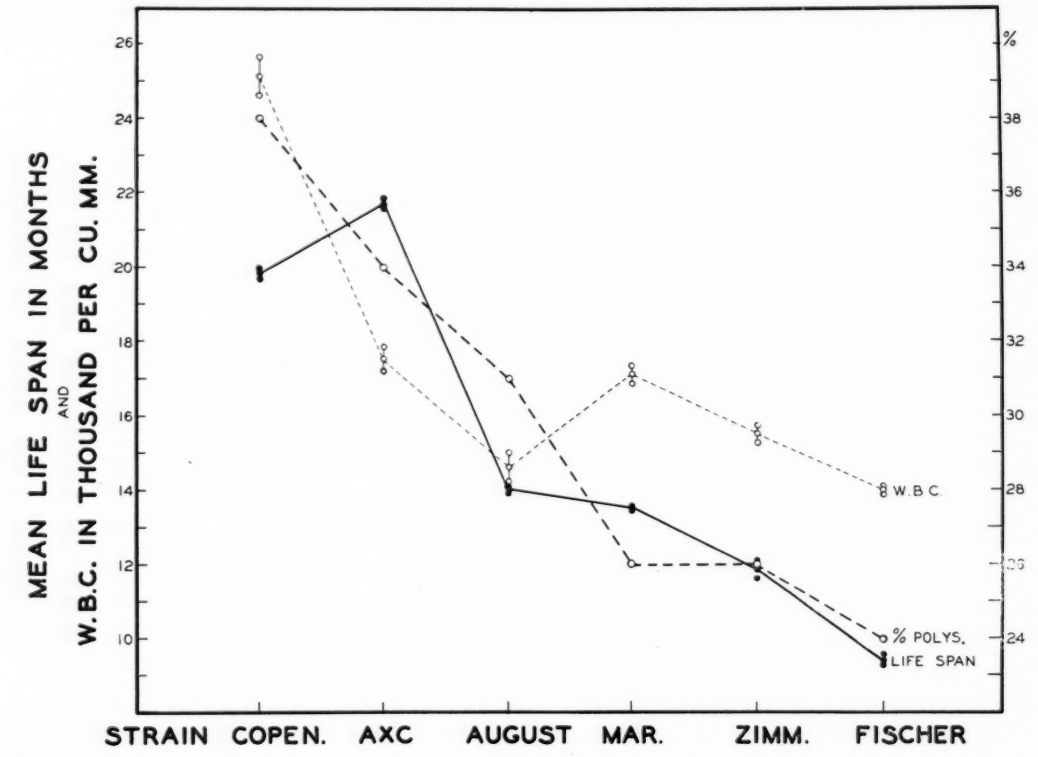

FIG. 3.—Relation of the mean total leucocyte count and percentage of neutrophil polymorphonuclears to the average life span in six inbred strains of rats.

3. Males tended to have higher hemoglobin values and red cell counts and lower white cell counts than females, but exceptions were observed in some strains.

4. Pregnant and lactating females had significantly lower hemoglobin values and red cell counts than normal females, but the white cell counts and differential values were little affected by either condition.

5. The maximum decrease in hemoglobin and in red cells was observed during the first 7 days after birth, and there was a return to normal at the beginning of the 4th week in females that were suckling young.

6. The strains with the longest life spans had the highest total leucocyte counts and the highest percentage of neutrophil polymorphonuclear leucocytes.

7. The strain with the lowest hemoglobin value had the highest spontaneous tumor history.

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Studies on the Morphology of the Peripheral Blood of Rats*

II. Rats Injected Subcutaneously with Carcinogenic Hydrocarbons

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A recent report (1) on the carcinogenic activity of methylcholanthrene in rats indicated that in addition to a higher cancer-inducing potency, methylcholanthrene had a considerably more toxic effect on rats than benzpyrene. Both chemicals were injected in various doses into the subcutaneous tissues of rats by a method previously described (2). Solid paraffin was used as the medium in order to secure a slow absorption of the active agent and make possible a study of the local response of the tissues to the maximal concentration of the injected material. The present study describes the changes resulting in the peripheral blood of rats from the localized areas of irritation.

The rats were bled from the tail vein under light ether anesthesia as described in the preceding paper of this series (4). Each was bled a few days before and at varying intervals after the injection.

TABLE I: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS OF RATS BEFORE INJECTION AND FROM 10 TO 30 DAYS AFTER INJECTION WITH VARIOUS DOSES OF METHYLCHOLANTHRENE, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	66	14.23 ± 0.10	8.05 ± 0.07	13.76 ± 0.35	34	60	4	1.5	0.03
10 days after 8 to 12 mgm.	66	14.64 ± 0.15	8.56 ± 0.09	14.49 ± 0.34	36	59	4	1.5	0.05
Difference		— 0.41 ± 0.18	— 0.51 ± 0.11	— 0.73 ± 0.49	— 2	1	0	0	— 0.02
Before injection	78	14.47 ± 0.08	8.00 ± 0.07	15.52 ± 0.50	40	54	4	1.7	0.01
20 days after 8 to 12 mgm.	78	14.49 ± 0.11	8.09 ± 0.08	18.13 ± 0.61	38	57	4	1.3	0.04
Difference		— 0.02 ± 0.14	— 0.09 ± 0.11	— 2.61 ± 0.79	2	— 3	0	0.5	— 0.03
Before injection	22	14.58 ± 0.18	8.00 ± 0.09	11.18 ± 0.55	32	64	3	0.7	0.09
30 days after 8 to 12 mgm.	22	14.85 ± 0.23	8.40 ± 0.20	15.86 ± 0.83	36	59	5	1.1	0.00
Difference		— 0.27 ± 0.29	— 0.40 ± 0.22	— 4.68 ± 1.0	— 4	5	— 2	— 0.4	0.09
Before injection	52	14.04 ± 0.16	7.80 ± 0.10	13.19 ± 0.45	32	62	4	1.5	0.04
30 days after 1 and 2 mgm.	52	14.75 ± 0.12	8.53 ± 0.07	16.92 ± 0.55	35	59	4	1.9	0.00
Difference		— 0.71 ± 0.20	— 0.73 ± 0.12	— 3.73 ± 0.71	— 3	3	0	0.4	0.04
Before injection	67	14.46 ± 0.11	8.00 ± 0.08	13.96 ± 0.38	34	60	4	1.8	0.04
30 days after 3 and 4 mgm.	67	14.78 ± 0.10	8.53 ± 0.08	17.36 ± 0.55	36	58	4	1.9	0.01
Difference		— 0.32 ± 0.15	— 0.53 ± 0.11	— 3.40 ± 0.67	— 2	2	0	— 0.1	0.03
Before injection	54	14.41 ± 0.10	8.09 ± 0.07	14.11 ± 0.54	38	56	4	1.7	0.00
30 days after 5 and 6 mgm.	54	14.56 ± 0.12	8.40 ± 0.09	16.56 ± 0.63	37	57	4	1.7	0.06
Difference		— 0.15 ± 0.16	— 0.31 ± 0.11	— 2.45 ± 0.83	1	1	0	0	0.06

The first three groups in Table I were injected with 8 to 12 mgm., the largest doses of methylcholanthrene administered. The 66 rats that were tested 10 days after the injection showed barely perceptible increases in the mean hemoglobin and in the mean red and white cell counts, with no change in the differential

* This study was undertaken and nearly completed under the direction of Dr. F. C. Wood at the Department of Cancer Research, Columbia University.

values. After 20 days 78 rats had no significant changes in any of these values, and after a 30 day interval 22 showed a small average increase in the white cell count. Unfortunately the majority of the rats died within the next 60 days without further testing. The 28 that survived from 60 to 140 days after the injection are presented in Table IV. They had just palpable early tumors, and an average decrease of nearly 3 gm. of hemoglobin and nearly a rats injected with 3 and 4 mgm. The average difference observed in the 54 injected with 5 and 6 mgm. was well within the experimental error.

Table II gives the average difference observed in three groups of rats tested 40 days after the injection of 1 and 2, 3 and 4, and 5 and 6 mgm. of methylcholanthrene respectively. In each group there was a significant decrease in the average hemoglobin values and in the red cell counts, and an increase in the white

TABLE II: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS OF RATS BEFORE INJECTION AND 40 DAYS AFTER INJECTION WITH VARIOUS DOSES OF METHYLCHOLANTHRENE, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	47	14.88 ± 0.12	9.06 ± 0.09	13.43 ± 0.38	28	66	4	1.6	0.00
40 days after 1 and 2 mgm.	47	12.97 ± 0.17	8.38 ± 0.13	15.04 ± 0.42	33	55	11	1.5	0.00
Difference		1.91 ± 0.21	0.68 ± 0.16	— 1.61 ± 0.57	— 5	11	— 7	0.2	0.00
Before injection	44	15.05 ± 0.10	8.99 ± 0.08	14.00 ± 0.41	31	63	5	1.4	0.00
40 days after 3 and 4 mgm.	44	13.05 ± 0.15	8.43 ± 0.11	16.27 ± 0.61	31	56	12	1.4	0.00
Difference		2.00 ± 0.18	0.56 ± 0.14	— 2.27 ± 0.73	0	7	— 7	0	0
Before injection	41	14.82 ± 0.13	8.62 ± 0.11	14.37 ± 0.44	26	67	5	1.9	0.00
40 days after 5 and 6 mgm.	41	12.43 ± 0.16	7.86 ± 0.14	16.71 ± 0.42	29	57	13	1.7	0.00
Difference		2.39 ± 0.21	0.76 ± 0.18	— 2.34 ± 0.61	—3	10	— 8	0.2	0.00

TABLE III: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS OF RATS BEFORE INJECTION AND 50 DAYS AFTER INJECTION WITH VARIOUS DOSES OF METHYLCHOLANTHRENE, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	58	15.22 ± 0.11	8.53 ± 0.06	14.86 ± 0.41	30	64	4	2.4	0.02
50 days after 1 and 2 mgm.	58	12.53 ± 0.11	8.45 ± 0.09	15.83 ± 0.43	31	57	9	2.9	0.00
Difference		2.69 ± 0.16	0.08 ± 0.11	— 0.97 ± 0.59	— 1	7	— 6	— 0.5	0.02
Before injection	54	15.33 ± 0.12	8.85 ± 0.08	15.52 ± 0.44	30	64	4	2.4	0.00
50 days after 3 and 4 mgm.	54	12.22 ± 0.13	8.39 ± 0.11	14.48 ± 0.45	33	55	10	1.9	0.00
Difference		3.11 ± 0.18	0.46 ± 0.14	1.04 ± 0.63	— 3	9	— 6	0.5	0.00
Before injection	41	15. 13 ± 0.14	8.52 ± 0.08	15.49 ± 0.51	29	64	4	2.9	0.00
50 days after 5 and 6 mgm.	41	12.04 ± 0.25	7.85 ± 0.19	14.17 ± 0.52	29	59	9	2.2	0.00
Difference		3.09 ± 0.29	0.67 ± 0.21	1.32 ± 0.73	0	5	— 5	0.7	0

million red cells per cu. mm. and an increase in the total white cell count that included a more than 200 per cent increase in the monocytes.

Rats injected with smaller doses of methylcholanthrene survived for further testing and perhaps indicate more clearly the sequence of the observed changes. From Table I it may be seen that 52 injected with 1 and 2 mgm. of methylcholanthrene had in a 30 day period a significant increase in mean hemoglobin value and in red and white cell counts, with little change in the differential values. During the same interval a slightly smaller average increase was observed for 67 cell counts that included a noteworthy increase in the percentage of monocytes.

Another series of rats injected with similar doses of methylcholanthrene were examined after 50 days. Table III indicates that the average hemoglobin values for these rats were reduced even further. The differences in red cell counts were less definite and perhaps for the smaller doses represent compensating regrowth of the red cells. The white cell counts were not significantly different, but the percentage of monocytes was consistently high.

The methylcholanthrene was injected subcutane-

ously in discrete masses of known volume, and when the first perceptible increase in size was detected another blood sample was taken. The progressive growth of the tumors was checked by further observations, the differences represent the changes observed in these values since the control tests made prior to injection with methylcholanthrene.

The rats in Table IV developed tumors between the

TABLE IV: SHOWING, FOR RATS THAT DEVELOPED METHYLCHOLANTHRENE TUMORS IN 60 TO 140 DAYS, THE MEAN HEMOGLOBIN, AND THE MEAN RED AND WHITE CELL COUNTS BEFORE INJECTION AND WHEN THE TUMORS WERE DISCOVERED, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	55	15.15 ± 0.14	8.86 ± 0.09	14.45 ± 0.46	31	63	4	1.6	0.05
Early tumors, 1 to 4 mgm.	55	13.28 ± 0.15	8.39 ± 0.12	18.49 ± 0.84	37	50	12	1.5	0.00
Difference		1.87 ± 0.21	0.47 ± 0.15	— 4.04 ± 0.96	— 6	13	— 8	0.1	0.05
Before injection	50	14.82 ± 0.12	8.47 ± 0.09	14.76 ± 0.45	28	65	4	2.3	0.00
Early tumors, 5 and 6 mgm.	50	12.58 ± 0.24	7.81 ± 0.17	15.64 ± 0.59	36	51	12	1.4	0.00
Difference		2.24 ± 0.27	0.66 ± 0.19	— 0.88 ± 0.74	— 8	15	— 8	0.9	0
Before injection	28	14.43 ± 0.14	8.09 ± 0.10	13.86 ± 0.48	39	56	4	1.3	0.07
Early tumors, 8 to 12 mgm.	28	11.54 ± 0.30	7.20 ± 0.23	16.93 ± 0.87	35	52	12	1.1	0.00
Difference		2.89 ± 0.33	0.89 ± 0.25	— 3.07 ± 0.99	— 4	4	— 8	0.2	0.07
Before injection	133	14.88 ± 0.08	8.55 ± 0.06	14.44 ± 0.27	32	62	4	1.8	0.04
Early tumors, 1 to 12 mgm.	133	12.65 ± 0.13	7.92 ± 0.10	17.09 ± 0.46	36	51	12	1.4	0.00
Difference		2.23 ± 0.15	0.63 ± 0.12	— 2.65 ± 0.52	— 4	12	— 8	0.4	0.04

TABLE V: SHOWING, FOR RATS THAT DEVELOPED METHYLCHOLANTHRENE TUMORS IN 140 TO 300 DAYS, THE MEAN HEMOGLOBIN, AND THE MEAN RED AND WHITE CELL COUNTS BEFORE INJECTION AND WHEN THE TUMORS WERE DISCOVERED, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	33	14.86 ± 0.11	8.58 ± 0.10	14.09 ± 0.47	30	64	4	2.1	0.00
Early tumors, 1 mgm.	33	12.53 ± 0.27	8.16 ± 0.17	16.64 ± 0.65	36	56	6	1.8	0.00
Difference		2.33 ± 0.29	0.42 ± 0.20	— 2.55 ± 0.80	— 6	8	— 3	0.3	0
Before injection	47	14.41 ± 0.16	8.10 ± 0.09	14.02 ± 0.48	30	64	3	2.1	0.02
Early tumors, 2 mgm.	47	13.16 ± 0.17	8.57 ± 0.13	19.47 ± 0.86	35	55	9	1.7	0.00
Difference		1.25 ± 0.23	— 0.47 ± 0.16	— 5.45 ± 0.98	— 5	9	— 5	0.3	0.02
Before injection	48	14.58 ± 0.12	8.50 ± 0.08	13.79 ± 0.43	31	63	4	2.0	0.00
Early tumors, 3 and 4 mgm.	48	12.87 ± 0.20	8.32 ± 0.15	17.87 ± 0.77	34	54	10	1.5	0.02
Difference		1.71 ± 0.23	0.18 ± 0.17	— 4.08 ± 0.88	— 3	8	— 6	0.5	— 0.02
Before injection	29	14.60 ± 0.14	8.51 ± 0.09	13.55 ± 0.61	33	61	4	1.9	0.00
Early tumors, 5 to 10 mgm.	29	13.64 ± 0.18	8.89 ± 0.16	20.66 ± 1.3	36	52	10	1.2	0.00
Difference		0.96 ± 0.23	— 0.38 ± 0.18	— 7.11 ± 1.4	— 3	9	— 6	0.8	0.00
Before injection	157	14.60 ± 0.07	8.40 ± 0.05	13.88 ± 0.25	31	63	4	2.0	0.01
Early tumors, 1 to 10 mgm.	157	13.03 ± 0.10	8.47 ± 0.08	18.61 ± 0.45	35	54	9	1.6	0.01
Difference		1.57 ± 0.12	— 0.07 ± 0.09	— 4.73 ± 0.51	— 4	9	— 5	0.5	0

and after autopsy diagnoses were made on the basis of the microscopic findings. The classification of the morphology and histogenesis of the tumors has been separately reported (1). The mean values in Table IV were obtained from blood tests of rats suspected of early malignant disease whose subsequent history proved the suspicion to have been well founded; and 60th and the 140th day after injection, and those in Table V developed them 140 to 320 days after injection. In both groups there was a significant decrease in the mean hemoglobin value. The average red cell count was reduced in the rats that developed tumors during the shorter latent period, but not in those that developed tumors later. In general the dose

of the carcinogen was larger for the former group, but the higher values in the latter group probably represent a regeneration during the longer interval. Fig. 1 indicates that the average hemoglobin was lowest about 50 days after injection and somewhat higher in rats tested later. The mean white cell counts were slightly increased in both groups, with a slight increase in the percentage of polymorphonuclear leucocytes and an increase of nearly 100 per cent or more in the monocytes. The differences in the percentage of monocytes are illustrated in Fig. 2.

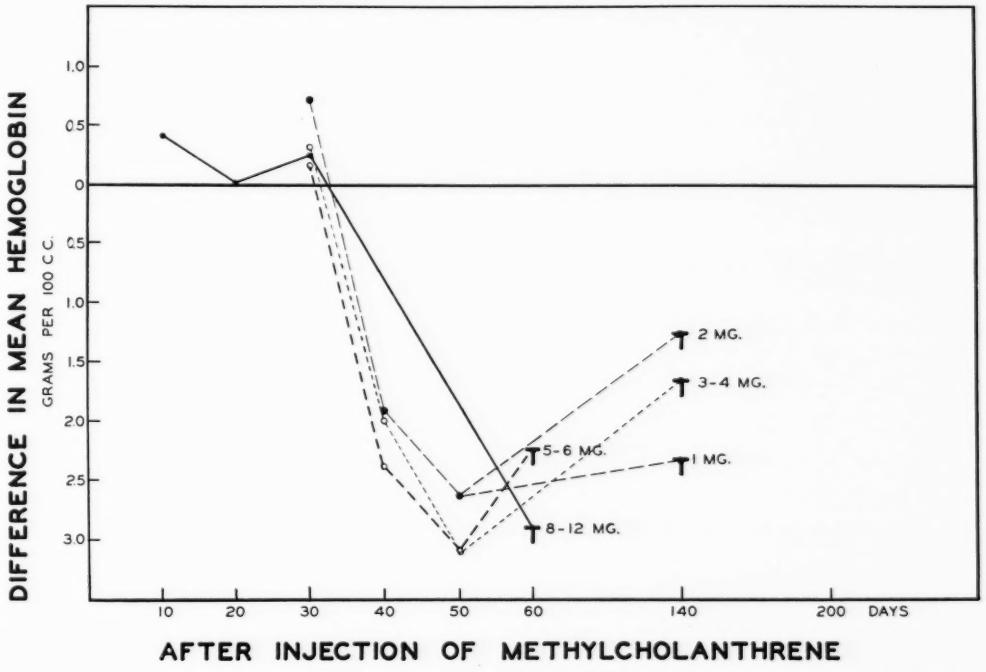

FIG. 1.—Changes in the mean hemoglobin value following the injection of various doses of methylcholanthrene.

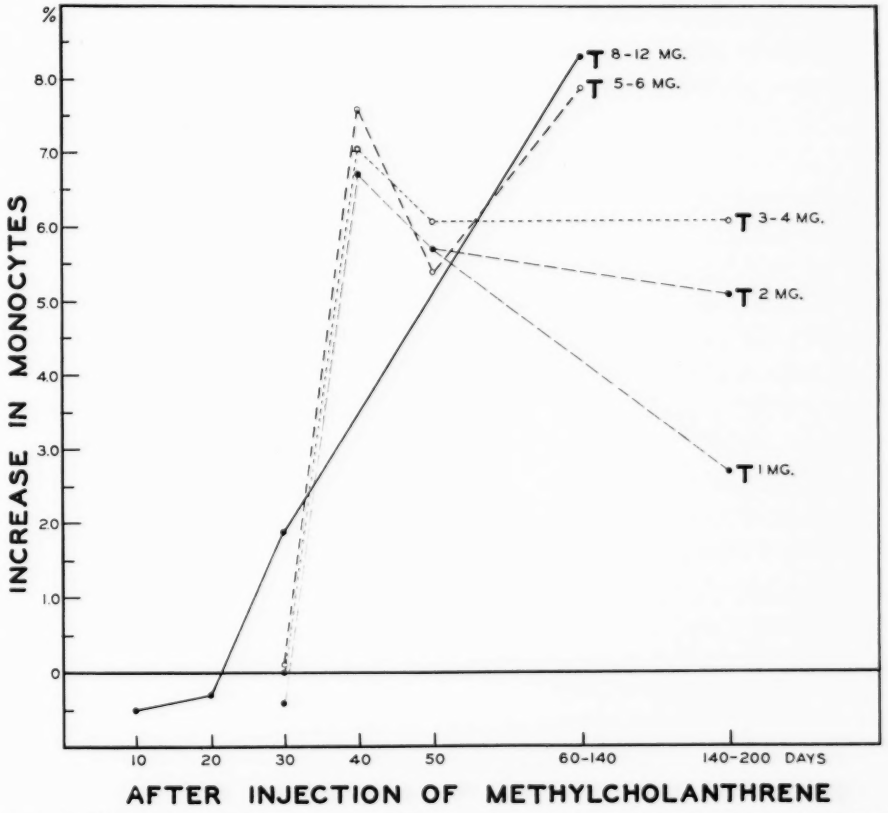

FIG. 2.—Increases in the percentage of monocytes following the injection of various doses of methylcholanthrene.

Similar tests were made on rats before and after

injection with 3,4-benzpyrene. The desirability of obtaining comparative information on the latent period in carcinogenesis for small doses of benzpyrene determined the experimental procedure, and this second series was injected with doses smaller and weaker than the doses of methylcholanthrene (3). For purposes of comparison, however, the largest doses in this series overlap the smaller doses of methylcholanthrene. Table VI shows that 0.2 to 1.0 mgm. of benzpyrene had in 30 to 35 days little or no effect on the mean hemoglobin value and that during this interval the mean red cell counts were slightly, and the percentage of monocytes notably, increased. The increase in percentage of monocytes was observed earlier than in other series after the injection of even larger doses of methylcholanthrene.

jected with 2 and 3 mgm. of benzpyrene showed no change in mean hemoglobin but a significant increase in the mean number of red cells. In both groups the mean white cell count was slightly increased and the percentage of monocytes consistently high. During a similar period (Table II) 1 and 2 mgm. of methylcholanthrene significantly decreased both the mean hemoglobin value and the mean number of red cells.

Early benzpyrene tumors developed in most of the rats after a somewhat longer latent period than was observed for the methylcholanthrene tumors. In Table VIII the benzpyrene tumors are recorded in three groups according to the length of the latent period, 60 to 200, 200 to 300, and over 300 days. Those in the first two groups are subdivided according to the dose of benzpyrene, and the third group includes mostly those with the weakest doses; *i.e.,* less than 1 mgm. In each group there was a significant decrease in the mean hemoglobin value and, with one exception, no change in the red cell count. The mean white

TABLE VI: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS OF RATS BEFORE INJECTION AND 30 TO 40 DAYS AFTER INJECTION WITH 3,4-BENZPYRENE, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	99	14.38 ± 0.10	8.96 ± 0.06	15.00 ± 0.36	31	61	6	1.7	0.01
30 days after 0.2 to 1.0 mgm.	99	13.83 ± 0.10	9.26 ± 0.06	16.92 ± 0.43	34	53	11	1.7	0.00
Difference		0.55 ± 0.14	— 0.30 ± 0.09	— 1.92 ± 0.55	— 3	8	— 4	0	0.01
Before injection	104	13.40 ± 0.10	8.57 ± 0.06	17.94 ± 0.48	32	60	6	2.5	0.00
35 days after 0.2 to 1.0 mgm.	104	13.85 ± 0.11	8.97 ± 0.08	20.37 ± 0.63	34	53	11	2.2	0.00
Difference		— 0.45 ± 0.15	— 0.40 ± 0.10	— 2.43 ± 0.79	— 1	7	— 6	0.3	0
Before injection	75	14.23 ± 0.10	8.75 ± 0.07	14.76 ± 0.33	21	71	6	2.3	0.00
40 days after 0.2 to 1.0 mgm.	75	14.22 ± 0.13	9.22 ± 0.07	16.17 ± 0.37	27	62	9	2.1	0.00
Difference		0.01 ± 0.16	— 0.47 ± 0.10	— 1.41 ± 0.50	— 6	9	— 3	0.2	0

TABLE VII: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS OF RATS BEFORE INJECTION AND 30 TO 40 DAYS AFTER INJECTION WITH 3,4-BENZPYRENE, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	77	13.98 ± 0.10	8.63 ± 0.06	16.43 ± 0.50	30	62	6	2.4	0.01
30 days after 1.0 to 2.0 mgm.	77	13.67 ± 0.15	9.10 ± 0.09	19.85 ± 0.64	33	54	11	2.5	0.00
Difference		0.31 ± 0.18	— 0.47 ± 0.11	— 3.42 ± 0.81	— 3	8	— 5	— 0.1	0.01
Before injection	130	14.42 ± 0.07	8.79 ± 0.06	14.97 ± 0.29	20	71	6	2.7	0.01
40 days after 1.0 to 2.0 mgm.	130	14.52 ± 0.09	9.20 ± 0.06	17.29 ± 0.37	26	63	9	2.0	0.00
Difference		— 0.10 ± 0.11	— 0.41 ± 0.08	— 2.32 ± 0.47	— 5	8	— 3	0.7	0.01
Before injection	183	14.49 ± 0.06	8.62 ± 0.04	15.46 ± 0.22	21	71	6	2.7	0.00
40 days after 2.0 to 3.0 mgm.	183	14.54 ± 0.07	9.25 ± 0.05	16.89 ± 0.29	25	63	10	2.1	0.02
Difference		— 0.05 ± 0.09	— 0.63 ± 0.06	— 1.43 ± 0.36	— 5	8	— 4	0.6	— 0.02

cell counts were slightly increased in all but the first group, which had the exceptionally decreased red cell count, and all showed some increase in the percentage of polymorphonuclear leucocytes and monocytes. Figures 3 and 4 reproduce graphically the changes in the mean hemoglobin value, and in the percentage of monocytes.

DISCUSSION

This study of the peripheral blood picture of rats injected subcutaneously with varying localized doses of methylcholanthrene or benzpyrene indicates that both carcinogens reduce the hemoglobin in two ways: first, by decreasing the number of red cells, and second, by decreasing the amount of hemoglobin in the cells.

TABLE VIII: MEAN HEMOGLOBIN, AND MEAN RED AND WHITE CELL COUNTS BEFORE INJECTION AND AFTER EARLY BENZPYRENE TUMORS WERE DISCOVERED, AND THE DIFFERENCES

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before injection	51	14.11 ± 0.13	8.71 ± 0.08	16.80 ± 0.55	28	65	5	2.2	0.00
Early tumors, 1 to 2 mgm. in 60 to 200 days	51	12.80 ± 0.19	8.08 ± 0.15	17.04 ± 0.71	39	53	7	1.5	0.00
Difference		1.31 ± 0.23	0.63 ± 0.17	— 0.24 ± 0.90	— 11	13	— 2	0.7	0
Before injection	44	14.34 ± 0.12	8.49 ± 0.09	15.91 ± 0.39	22	71	5	2.4	0.00
Early tumors, 2 to 3 mgm. in 60 to 200 days	44	13.45 ± 0.16	8.72 ± 0.13	18.14 ± 0.69	36	54	8	2.1	0.00
Difference		0.89 ± 0.20	— 0.23 ± 0.16	— 2.23 ± 0.79	— 14	17	— 3	0.3	0
Before injection	47	13.85 ± 0.16	8.55 ± 0.10	17.91 ± 0.77	30	63	5	2.4	0.00
Early tumors, 0.2 to 2.0 mgm. in 200 to 300 days	47	12.31 ± 0.19	8.59 ± 0.15	23.09 ± 1.2	41	50	7	1.7	0.00
Difference		1.54 ± 0.25	— 0.04 ± 0.18	— 5.18 ± 1.4	— 12	13	— 3	0.7	0
Before injection	44	14.30 ± 0.10	8.61 ± 0.08	15.23 ± 0.59	21	71	6	2.3	0.00
Early tumors, 2 to 3 mgm. in 200 to 300 days	44	12.36 ± 0.22	8.38 ± 0.14	17.45 ± 0.80	37	54	7	2.1	0.00
Difference		1.94 ± 0.24	0.23 ± 0.16	— 2.22 ± 0.99	— 16	17	— 1	0.2	0.00
Before injection	54	14.26 ± 0.12	8.60 ± 0.09	15.52 ± 0.40	22	70	6	2.0	0.02
Early tumors, 0.2 to 3.0 mgm. in 300 or more days	54	12.72 ± 0.20	8.56 ± 0.13	19.85 ± 0.91	42	48	8	1.9	0.00
Difference		1.54 ± 0.23	0.04 ± 0.16	— 4.33 ± 0.99	— 20	22	— 2	0.2	0.02

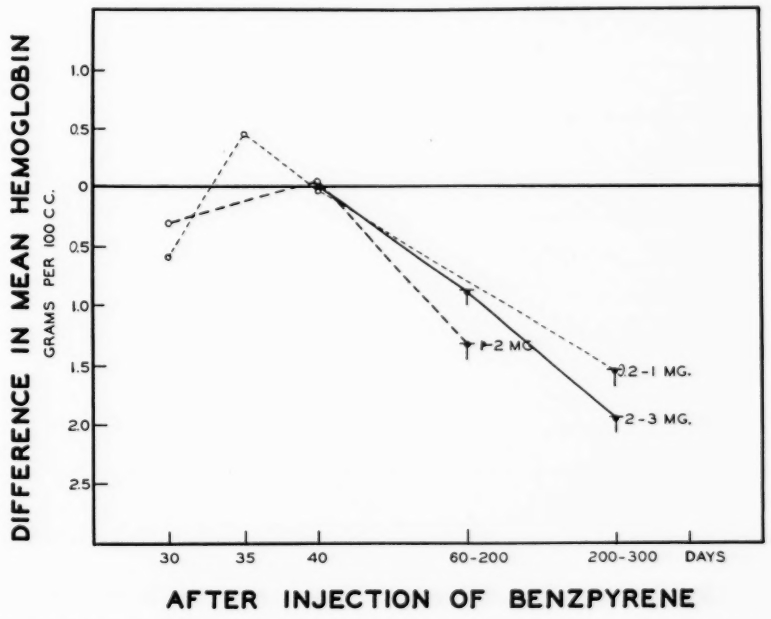

FIG. 3.—Changes in the mean hemoglobin value following the injection of various doses of benzpyrene.

No decrease in the hemoglobin was observed less than 40 days after injection even with the largest doses; *i.e.,* 8 to 12 mgm. of methylcholanthrene. Within 90 days after injection most of the rats that had received large doses were dead with enlarged, congested lymph nodes, livers, and spleens. Rats injected with smaller doses of methylcholanthrene showed in less than 40 days a slight but significant increase in the mean number of red cells and for the smallest dose of methylcholanthrene; *i.e.,* 1 to 2 mgm., a significant increase in hemoglobin also. Although the carcinogen was applied locally in insoluble nodules of paraffin, there was a systemic response expressed by an increased production of red cells. However, after 40 to 50 days this initial excitation was overcome, and the rats showed a decided decrease in both hemoglobin value and red cells. Presumably the animals most severely affected died and the somewhat less anemic developed tumors 60 days or more after injection.

Taylor and Pollack (5) reported that the precancerous condition induced in mice by methylcholanthrene was associated with a gradual fall in hemoglobin value. A gradual decrease in hemoglobin level is apparent from their chart, but the figures in their table show no real difference in the first group between the 50th and 83rd days or in the second group from the 84th to the 103rd day. The same authors found a drop in hemoglobin level in rats after the ingestion of *p*-dimethylaminoazobenzene for 30 days. The low level of hemoglobin was maintained over a 162 day period and was certainly significant for the rats on the rice and raw carrot diet. The decrease in hemoglobin value was less definite in rats on a Purina fox chow diet and the differences were not statistically significant.

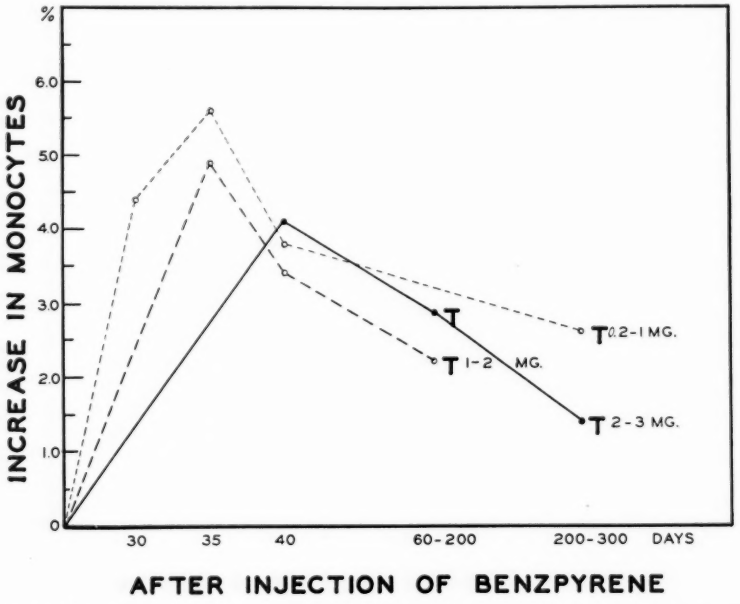

FIG. 4.—Increases in the percentage of monocytes following the injection of various doses of benzpyrene.

A significant increase in the number of red cells was observed in 30 to 50 days after the injection of 0.2 to 3.0 mgm. of benzpyrene, but this was not accompanied by a change in the hemoglobin value; thus it is indicated that the regeneration of red cells was able to compensate for the reduction of hemoglobin. These and larger doses of benzpyrene were not lethal, and most of the rats survived to develop tumors 60 to 400 days after injection. When the tumors were observed, the mean hemoglobin value was significantly decreased while the number of red cells did not differ significantly from the number observed at the time of injection. Therefore, there was a smaller amount of hemoglobin per cell.

With both carcinogens the percentage of monocytes showed a decided increase 40 days after injection and remained high as long as the observations were continued; *i.e.,* until the tumors appeared. These chemicals affect the blood in two ways: first, by direct action on the circulating cells, and second, by involving the formative tissue, the bone marrow. The action on the peripheral blood cells is hemolytic in type and results in the destruction of red cells. This red cell destruction in turn causes a compensatory stimulation of the bone marrow with an increased production of erythrocytes, smaller in size and containing less than the normal amount of hemoglobin per cell. This produces a microcytic hypochromic anemia.

When these agents act directly on the bone marrow they either stimulate or depress it, depending upon the concentration. Both exert a definite effect on the peripheral blood, as is shown by the production

of monocytes, and both act directly on the bone marrow, small amounts stimulating it and producing more cells and large amounts depressing cell formation. The peripheral hemolytic and central depressant action of these chemicals is not unusual, but the central stimulating effect is worthy of comment, as some investigators have been able to produce leukemia by utilizing this action over a long period of time.

The significance of the increase in monocytes is susceptible of wide speculation. It is well known that foreign body particles cause a monocytic response whose endeavor it is to remove the offending material. In the case of tumors induced by *Cysticercus fasciolaris,* monocytes appear about the encysted parasite and sometimes seem to take part in the tumor formation. Whether the early sarcoma-like cells are actually related to the monocyte or to its endothelial forerunner is uncertain; an increase in monocytes observed after a definite latent period in animals injected with carcinogenic chemicals suggests the possibility that the increase in monocytes may be related to the initiation of the malignant process. It is of further interest to consider whether or not the anemia produced by these compounds is analogous to the endemic anemia previously mentioned (4) in a strain of rats and a strain of mice each with a high incidence of spontaneous tumors.

SUMMARY

1. As previously reported, methylcholanthrene showed not only a higher carcinogenic potency but also a more toxic action when injected in solid paraffin into the subcutaneous tissues of rats than did benzpyrene. The present study records the changes observed in the peripheral blood of rats injected with varying doses of these two carcinogens.

2. The largest doses of methylcholanthrene, 8 to 12 mgm., were toxic, and most of the rats died within 90 days after injection. Ten days after the injection they showed a slight increase in hemoglobin value and in red and white cell counts but no change in the differential values. Twenty days after injection there was no significant change in any of these values, and after 30 days the only significant alteration observed was a slight increase in the white cell count. Unfortunately the only rats tested later were the ones in which early tumors had developed. At this time there was an average decrease in hemoglobin of nearly 3 gm. per 100 cc., a decrease of nearly a million red cells per cu. mm., and an increase in the total white cell count that included a more than 200 per cent increase in the monocytes.

3. Rats that received smaller (1 to 6 mgm.), less toxic, doses of methylcholanthrene survived for further testing and for the first 40 days showed a significant increase in the red and white cell counts, but after 40 to 50 days there was a significant decrease in the hemoglobin value and in the number of red cells, with no further increase in the white cell count. The percentage of monocytes, however, remained high. The blood picture when tumors were first observed, 60 to 320 days after injection, was similar except for a slight increase in the total white cell count.

4. Up to 50 days after the injection of benzpyrene rats showed no significant change in the hemoglobin value, but a significant increase in the number of red cells and the percentage of monocytes. By the time the tumors appeared there were significant decreases in hemoglobin and a return of the red cell counts to the preinjection level. A majority showed a slight increase in the total white cells, including an increase in the percentage of polymorphonuclear leucocytes and monocytes.

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Studies on the Morphology of the Peripheral Blood of Rats*

III. Rats with Induced and Transplanted Tumors

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The preceding reports in this series (11, 7) recorded studies of the peripheral blood pictures of normal, pregnant, and lactating rats of several inbred strains and of rats injected with two carcinogenic chemicals, methylcholanthrene and benzpyrene. The present report is based on a series of observations of rats with methylcholanthrene and benzpyrene tumors and of rats with progressively growing transplanted tumors of diverse origin. The material and methods used are described in the first two papers. There were 223 rats with one or more tumors induced by methylcholanthrene, and 190 with benzpyrene tumors. The peripheral blood picture of each was obtained before injection, 40 to 70 days after injection, at the first appearance of the tumor, and after the tumor was advanced but before ulceration occurred. The rats with tumors were divided into four groups according to the carcinogen and the dose. The data obtained are presented in Tables I to IV. Table V gives the average age for each group and the average interval between the first and subsequent blood tests.

Table I includes the data on 101 tumor rats that had been injected with 1 to 3 mgm. of methylcholanthrene. The mean values before injection were: hemoglobin 14.8 gm. per 100 cc., and red cells 8.6 million and white cells 14.2 thousand per cu. mm. The differential values were: 29 per cent polymorphonuclear neutrophil leucocytes, 65 per cent lymphocytes, 4 per cent monocytes, and 2 per cent eosinophils.

TABLE I: PERIPHERAL BLOOD PICTURES OF RATS BEFORE AND AFTER TUMORS WERE INDUCED BY 1 TO 3 MGM. OF METHYLCHOLANTHRENE

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Control	101	14.82 ± 0.09	8.56 ± 0.06	14.15 ± 0.30	29	65	4	2.1	0.03
Carcinogen, 1 to 3 mgm. of M-C	101	13.07 ± 0.10	8.50 ± 0.07	16.12 ± 0.38	33	57	9	2.1	0.00
Difference		1.75 ± 0.13	0.06 ± 0.09	— 1.97 ± 0.46	— 3	8	— 5	0	0.03
Early tumors	101	13.22 ± 0.11	8.54 ± 0.08	18.47 ± 0.55	36	54	9	1.5	0.01
Difference from control		1.60 ± 0.14	0.02 ± 0.10	— 4.32 ± 0.63	— 6	11	— 5	0.6	0.02
Difference from carcinogen		— 0.15 ± 0.15	— 0.04 ± 0.11	— 2.35 ± 0.67	— 3	3	0	0.6	0.01
Advanced tumors	101	10.65 ± 0.17	6.48 ± 0.12	24.43 ± 0.85	51	39	8	1.2	0.00
Difference from control		4.17 ± 0.19	2.08 ± 0.13	— 10.28 ± 0.90	— 22	26	— 5	0.9	0.03
Difference from carcinogen		2.42 ± 0.20	2.02 ± 0.14	— 8.31 ± 0.93	— 19	18	0	0.9	0
Difference from early tumors		2.57 ± 0.20	2.06 ± 0.14	— 5.96 ± 1.0	— 16	15	0	0.3	— 0.01

After the injection of methylcholanthrene there was a significant decrease of 1.75 ± 0.13 gm. of hemoglobin with no change in the red cell count and only a slight increase, 1.97 ± 0.46 thousand, in the white cells, which included a 125 per cent increase in the percentage of monocytes. When the tumors were first observed, 190 days after the preinjection blood test, as shown in Table V, this blood picture persisted except for a slight increase (2.35 ± 0.67 thousand per cu. mm.) in the total white cell count. When the tumors had reached an advanced stage, the hemoglobin had dropped to 10.17 ± 0.17 gm., the red cell count to 6.5 ± 0.12 million

* This study was undertaken and nearly completed under the direction of Dr. F. C. Wood at the Department of Cancer Research, Columbia University.

per cu. mm., and the total white cell count had increased to 24.4 ± 0.85 thousand. The percentage of polymorphonuclear leucocytes rose to 51; the percentage of lymphocytes dropped to 39; and the percentage of monocytes remained at the high level attained after the injection of the carcinogen.

The data on 122 tumor rats that had been injected with 4 to 10 mgm. of methylcholanthrene are presented in Table II. The respective blood pictures were essentially the same as those observed with the smaller doses. The rats in the second group had before injection a mean hemoglobin value of 14.6 gm. per 100 cc., and 8.4 million red cells and 14.1 thousand white cells per cu. mm. The differential values were 33 per cent polymorphonuclear neutrophil leucocytes, 61 per cent lymphocytes, 4 per cent monocytes, and 2 per cent eosinophils. After injection of the carcinogen the hemoglobin value was reduced by 1.15 ± 0.13 gm., the number of red cells remained unchanged, and white cell count was increased by 2.57 ± 0.42 thousand per cu. mm. The only alteration in the differential values was a shift of 4 per cent from lymphocytes to monocytes. When the tumors were first discovered the blood picture was essentially the same, except for a further increase in the percentage of monocytes. When the growths had become advanced, an average of 180 days after the first blood test, the mean hemoglobin was reduced by 4.99 ± 0.19 gm. per 100 cc., the red cell count by 2.58 ± 0.13 million per cu. mm., while the total white cell count was increased by 14.13 ± 1.1 thousand per cu. mm. The differential values showed an increase of 21 per cent in the polymorphonuclear neutrophil leucocytes and of 5 per cent in the monocytes, accompanied by a decrease of 24 per cent in the lymphocytes.

Somewhat smaller doses of benzpyrene produced similar changes, but the latent period before the occurrence of the tumors was considerably longer (Table V). The 101 rats with tumors induced by 0.2 to 2 mgm. of benzpyrene had before injection a mean hemoglobin value of 14.1 gm., a red cell count of 8.7 million, and a white cell count of 17 thousand.

TABLE II: PERIPHERAL BLOOD PICTURES OF RATS BEFORE AND AFTER TUMORS WERE INDUCED BY 4 TO 10 MGM. OF METHYLCHOLANTHRENE

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Control	122	14.62 ± 0.08	8.38 ± 0.06	14.13 ± 0.30	33	61	4	2.0	0.01
Carcinogen, 4 to 10 mgm. of M-C	122	13.47 ± 0.10	8.25 ± 0.07	16.70 ± 0.29	33	57	8	1.8	0.02
Difference		1.15 ± 0.13	0.13 ± 0.09	— 2.57 ± 0.42	0	4	— 4	0.3	— 0.01
Early tumors	122	13.10 ± 0.11	8.39 ± 0.09	18.23 ± 0.51	35	53	11	1.3	0.00
Difference from control		1.52 ± 0.14	— 0.01 ± 0.11	— 4.10 ± 0.59	— 2	8	— 7	0.7	0.01
Difference from carcinogen		0.37 ± 0.15	— 0.14 ± 0.11	— 1.53 ± 0.59	— 2	5	— 3	0.4	0.02
Advanced tumors	122	9.63 ± 0.17	5.80 ± 0.12	28.26 ± 1.0	53	37	9	0.7	0.01
Difference from control		4.99 ± 0.19	2.58 ± 0.13	— 14.13 ± 1.1	— 21	24	— 5	1.3	0
Difference from carcinogen		3.84 ± 0.20	2.45 ± 0.14	— 11.56 ± 1.1	— 21	21	— 1	1.1	0.02
Difference from early tumors		3.47 ± 0.20	2.59 ± 0.15	— 10.03 ± 1.2	— 18	16	2	0.6	— 0.01

TABLE III: PERIPHERAL BLOOD PICTURES OF RATS BEFORE AND AFTER TUMORS WERE INDUCED BY 0.2 TO 2.0 MGM. OF BENZPYRENE

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Control	101	14.07 ± 0.09	8.68 ± 0.06	17.00 ± 0.45	27	66	5	2.5	0.01
Carcinogen, 0.2 to 2.0 mgm. of B-P	101	13.98 ± 0.11	9.25 ± 0.07	20.47 ± 0.59	31	57	10	2.2	0.00
Difference		0.09 ± 0.14	— 0.57 ± 0.09	— 3.47 ± 0.74	— 4	9	— 5	0.2	0.01
Early tumors	101	12.71 ± 0.14	8.46 ± 0.11	20.78 ± 0.69	41	50	7	1.8	0.00
Difference from control		1.36 ± 0.17	0.22 ± 0.13	— 3.78 ± 0.82	— 14	16	— 2	0.8	0.01
Difference from carcinogen		1.27 ± 0.18	0.79 ± 0.13	— 0.31 ± 0.91	— 10	8	2	0.5	0
Advanced tumors	101	9.64 ± 0.19	6.31 ± 0.12	34.90 ± 1.5	58	34	7	1.3	0.00
Difference from control		4.43 ± 0.21	2.37 ± 0.13	— 17.90 ± 1.6	— 31	32	— 2	1.2	0.01
Difference from carcinogen		4.34 ± 0.22	2.94 ± 0.14	— 14.43 ± 1.6	— 27	23	— 2	0.9	0
Difference from early tumors		3.07 ± 0.24	2.15 ± 0.16	— 14.12 ± 1.7	— 16	16	0	0.4	0

The differential values were 27 per cent polymorphonuclear neutrophil leucocytes, 66 per cent lymphocytes, 5 per cent monocytes, and 2 per cent eosinophils. After the injection of benzpyrene there was no perceptible change in the hemoglobin value, but there was a significant increase, 0.57 ± 0.09 million per cu. mm., in the red cell count. The white cell count was increased by 3.47 ± 0.74 thousand. The differential values showed an increase of 4 per cent in polymorphonuclear leucocytes and of 5 per cent in monocytes. When the tumors were first observed, an average of 263 days after the first blood test (Table V), the average hemoglobin value was significantly reduced, and the average red cell count had decreased to a little below the preinjection level. The rats with late tumors showed a further reduction in the hemoglobin and in the red cell count and a notable increase in the total white cell count that included a 31 per cent increase in neutrophil polymorphonuclear leucocytes. Somewhat larger doses of benzpyrene and the resultant tumors produced the same general effect on the peripheral blood (Table IV).

Tables I to IV record the changes in the several elements of the peripheral blood following the injection of larger and smaller doses of each of the carcinogens and the presence of early and advanced tumors.

Figs. 1 to 5 show graphically the changes in hemoglobin, red and white cell counts, and percentage of polymorphonuclear leucocytes and monocytes. In Fig. 1 it may be seen that large and small doses of methylcholanthrene reduced the mean hemoglobin value prior to the occurrence of the tumors, and that the doses of benzpyrene administered brought about no change in the hemoglobin value until the tumors had appeared. Fig. 2 shows that in the rats injected with benzpyrene the hemoglobin value was probably maintained at the normal level after injection of the chemical by an increase in the number of red cells. The significant decrease in hemoglobin when the tumors were first observed could not be attributed to a decrease in the number of red cells, but continued growth of the tumor definitely reduced both the red cell count and the hemoglobin value. The mean total white cell count (Fig. 3) increased slightly after the injection of both carcinogens and at the appearance of the tumors but increased much more rapidly with the continued growth of the neoplasms. The percentage of polymorphonuclear leucocytes (Fig. 4) increased similarly.

Table V shows that at the time of the final blood test the rats of the several groups averaged from 436 to 500 days of age. The increase in the total white cells and in the percentage of polymorphonuclear leucocytes in these rats with advanced tumors was well above the normal level for this age. In a majority of cases the autopsy findings included evidence of respiratory infections, which were probably terminal but which may have elevated the counts slightly. None of the animals gave evidence of leukemia or had

TABLE IV: PERIPHERAL BLOOD PICTURES OF RATS BEFORE AND AFTER TUMORS WERE INDUCED BY 2 AND 3 MGM. OF BENZPYRENE

Group	Number of rats	Mean + P.E. hemoglobin, gm.	Mean + P.E. R.B.C., millions	Mean + P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Control	89	14.39 ± 0.08	8.53 ± 0.06	15.49 ± 0.32	21	71	6	2.3	0.00
Carcinogen, 2 to 3 mgm. of B-P	89	14.52 ± 0.09	9.22 ± 0.07	16.06 ± 0.32	25	63	9	2.1	0.02
Difference		-0.13 ± 0.12	-0.69 ± 0.09	-0.57 ± 0.44	−4	8	−4	0.2	−0.02
Early tumors	89	13.07 ± 0.12	8.62 ± 0.09	17.27 ± 0.47	37	54	8	2.0	0.00
Difference from control		1.32 ± 0.14	-0.09 ± 0.11	-1.78 ± 0.57	−16	18	−2	0.2	0
Difference from carcinogen		1.45 ± 0.15	0.60 ± 0.11	-1.21 ± 0.57	−11	9	2	0.1	0.02
Advanced tumors	89	10.06 ± 0.20	6.54 ± 0.13	28.44 ± 1.2	56	35	8	1.1	0.00
Difference from control		4.33 ± 0.22	1.99 ± 0.14	-12.95 ± 1.3	−35	37	−2	1.2	0
Difference from carcinogen		4.46 ± 0.22	2.68 ± 0.15	-12.38 ± 1.3	−31	28	1	1.0	0.02
Difference from early tumors		3.01 ± 0.23	2.08 ± 0.16	-11.17 ± 1.3	−19	19	0	0.9	0

TABLE V: AVERAGE AGE OF RATS AT EACH BLOOD TEST AND AVERAGE INTERVAL BETWEEN FIRST AND SUBSEQUENT TESTS

Group	Number of rats	Age, days	Difference
Control	101	243 ± 4.8	
Carcinogen, 1 to 3 mgm. of M-C		309 ± 4.9	65
Early tumors		433 ± 6.1	190
Advanced tumors		456 ± 5.6	213
Control	122	267 ± 5.4	
Carcinogen, 4 to 10 mgm. of M-C		315 ± 5.0	48
Early tumors		420 ± 5.8	153
Advanced tumors		447 ± 5.5	180
Control	101	193 ± 5.2	
Carcinogen, 0.2 to 2 mgm. of B-P.		262 ± 6.0	69
Early tumors		456 ± 7.5	263
Advanced tumors		500 ± 7.5	307
Control	89	154 ± 4.7	
Carcinogen, 2 to 3 mgm. of B-P		211 ± 3.3	57
Early tumors		389 ± 5.8	235
Advanced tumors		436 ± 6.1	282

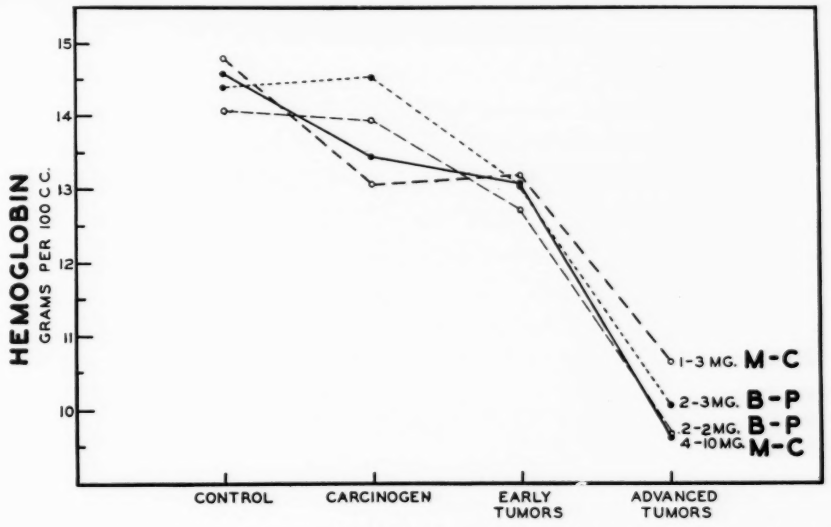

FIG. 1.—Mean hemoglobin value for rats before and after tumors were induced by various doses of methylcholanthrene or benzpyrene.

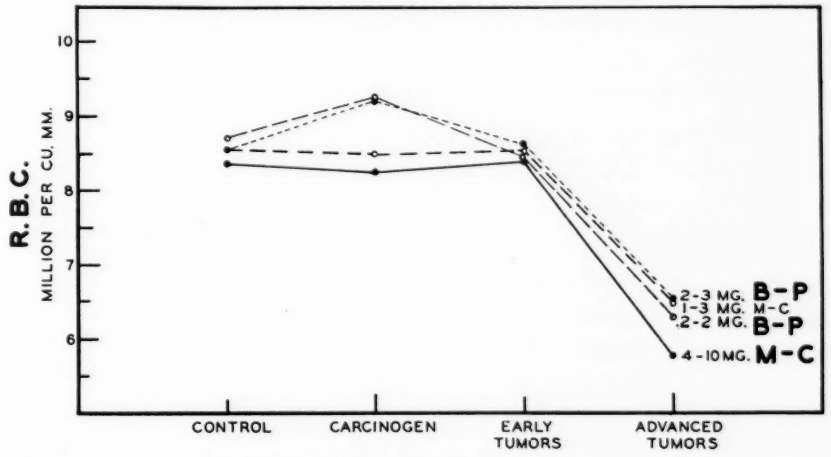

FIG. 2.—Mean red cell count for rats before and after tumors were induced by various doses of methylcholanthrene or benzpyrene.

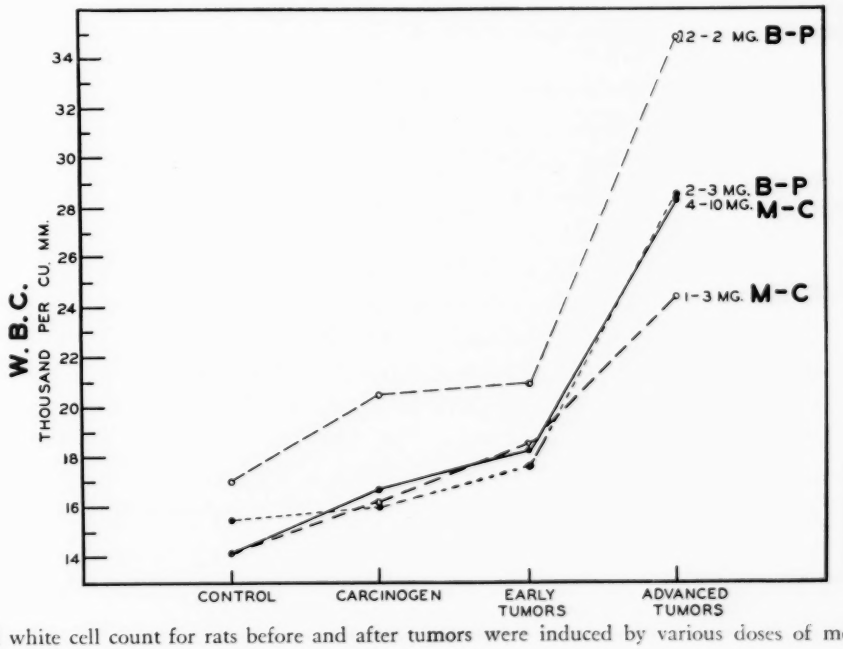

FIG. 3.—Mean total white cell count for rats before and after tumors were induced by various doses of methylcholanthrene or benzpyrene.

counts high enough to be considered preleukemic. Leukemia was not induced in any rats of this series and occurs but rarely in any of these stocks. When the tumors were first discovered the rats varied in age from 389 to 456 days, but their peripheral blood picture, except for a slight increase in polymorphonuclear leucocytes, was characteristic of that previously observed (11) for these rats at about 50 days of age.

The increase in the percentage of monocytes (Fig. 5) seems to be a specific effect of the carcinogens that is maintained throughout the first appearance of the tumors and their continued growth.

Changes in the peripheral blood due to the continuous growth of induced tumors may be compared with those caused by the progressive growth of transplanted tumors. For this comparison rat sarcomas of several origins were used. Some of these, *Cysticercus* sarcomas 1548, 4337, and 6820, and spontaneous tumors 92 and 95, were transplanted into the closely inbred strains in which the primary tumors arose, and others, J.R.S. and I.R.S. 146, were transplanted into closely inbred and well adapted strains not related to the host of the primary tumor. This study was confined to rats with progressively growing neoplasms, and the changes observed in the peripheral blood were not elicited by the so called immunity or foreign tissue reactions which prevent tumor growth.

One of the seven sarcomas used in this investigation was the Jensen rat sarcoma, which grew progressively in the rats of line 520 of the Marshall strain. The other six were from primary growths that occurred spontaneously—R 92 (2) and R 95 (4)—or were induced by *Cysticercus*—I.R.S. 146, 1548 (3), 4337 (6, 9), and 6820 (5)—in rats of the pedigreed colony at the Department of Cancer Research of Columbia University. Two blood tests were obtained for each

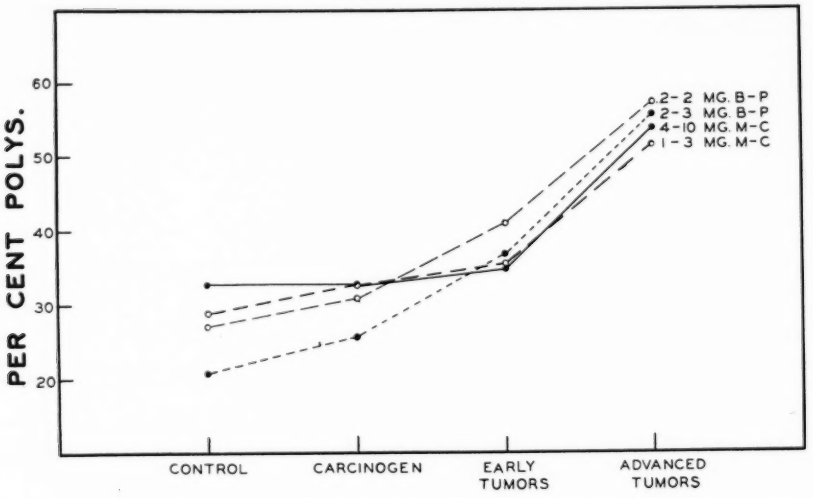

FIG. 4.—Average percentage of polymorphonuclear neutrophil leucocytes in rats before and after tumors were induced by various doses of methylcholanthrene or benzpyrene.

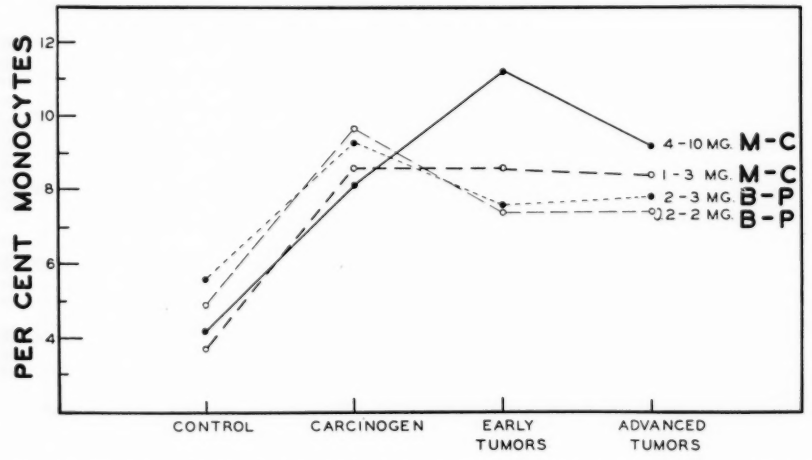

FIG. 5.—Average percentage of monocytes in rats before and after tumors were induced by various doses of methylcholanthrene or benzpyrene.

rat, the first before inoculation and the other after the tumor had attained considerable size but before ulceration occurred (Table VI).

The progressive growth of all the tumors studied decreased significantly the mean hemoglobin value. The red cell count was significantly depressed in all but bearers of I.R.S. 1548 and 146. The mean white cell count was significantly increased in all except bearers of R 92. In each case there was an increase in the percentage of polymorphonuclear leucocytes with adenocarcinoma implants the hemoglobin level was decreased by the time the tumor had attained measurable size, and that it fell progressively until death.

Table VII shows for each of the tumors considered in the present report the interval between its inoculation and the second blood test and the average daily gain in diameter. All the transplanted tumors were of considerable size as were the advanced induced tumors and, like the large spontaneous and trans-

TABLE VI. PERIPHERAL BLOOD PICTURE OF RATS BEFORE AND AFTER THE PROGRESSIVE GROWTH OF TRANSPLANTED SARCOMAS

Group	Number of rats	Mean ± P.E. hemoglobin, gm.	Mean ± P.E. R.B.C., millions	Mean ± P.E. W.B.C., thousands	Polymorphonuclear leucocytes, per cent	Lymphocytes, per cent	Monocytes, per cent	Eosinophils, per cent	Basophils, per cent
Before inoculation	48	12.90 ± 0.10	8.57 ± 0.08	22.74 ± 1.1	34	57	6	3.1	0.00
Positive I.R.S. 4337	48	9.81 ± 0.27	6.81 ± 0.20	29.50 ± 1.1	42	49	6	3.2	0.00
Difference		3.09 ± 0.29	1.76 ± 0.22	− 6.76 ± 1.5	− 7	8	0	− 0.1	0
Before inoculation	205	14.05 ± 0.05	8.86 ± 0.05	13.71 ± 0.17	28	62	9	1.8	0.01
Positive I.R.S. 1548	205	13.40 ± 0.09	9.11 ± 0.07	15.79 ± 0.25	35	56	8	1.6	0.00
Difference		0.65 ± 0.10	− 0.25 ± 0.09	− 2.08 ± 0.30	− 7	6	1	0.2	0.01
Before inoculation	24	12.71 ± 0.19	8.50 ± 0.13	12.08 ± 0.47	20	70	7	2.7	0.00
Positive R 95	24	11.54 ± 0.28	7.77 ± 0.18	18.67 ± 0.68	38	50	8	4.3	0.00
Difference		1.17 ± 0.34	0.73 ± 0.22	− 6.59 ± 0.83	− 18	21	− 1	1.6	0
Before inoculation	45	13.14 ± 0.10	8.45 ± 0.09	16.47 ± 0.45	29	61	6	4.2	0.05
Positive I.R.S. 6820	45	12.30 ± 0.17	7.83 ± 0.15	32.02 ± 1.3	44	46	8	1.9	0.02
Difference		0.84 ± 0.20	0.62 ± 0.17	− 15.55 ± 1.4	− 15	15	− 2	2.4	0.03
Before inoculation	59	13.28 ± 0.13	8.87 ± 0.10	17.98 ± 0.47	22	69	7	2.0	0.00
Positive R 92	59	11.09 ± 0.17	7.22 ± 0.13	16.53 ± 0.58	33	56	7	3.1	0.00
Difference		2.19 ± 0.21	1.65 ± 0.16	1.45 ± 0.75	− 11	12	0	1.1	0
Before inoculation	32	13.88 ± 0.13	8.61 ± 0.15	11.69 ± 0.42	22	70	6	1.8	0.00
Positive I.R.S. 146	32	13.12 ± 0.18	8.46 ± 0.13	22.00 ± 0.93	39	50	10	0.8	0.00
Difference		0.76 ± 0.22	0.15 ± 0.20	− 10.31 ± 1.0	− 17	20	− 4	0.9	0
Before inoculation	18	13.78 ± 0.22	9.06 ± 0.19	17.89 ± 0.62	23	69	7	1.3	0.00
Positive J.R.S.	18	10.17 ± 0.44	6.83 ± 0.27	33.67 ± 2.1	44	46	9	1.4	0.00
Difference		3.61 ± 0.49	2.23 ± 0.33	− 15.78 ± 2.2	− 21	23	− 2	0.1	0
Total before inoculation	431	13.63 ± 0.04	8.76 ± 0.03	15.49 ± 0.20	27	64	7	2.2	0.01
Total positives	431	12.30 ± 0.08	8.24 ± 0.06	20.51 ± 0.33	37	53	8	2.1	0.02
Difference		1.32 ± 0.09	0.52 ± 0.07	− 5.02 ± 0.39	− 10	11	0	0.1	0.01

which was about equal to the reduction in the percentage of lymphocytes. In rats with I.R.S. 146 there was an increase of 4 per cent in the monocytes.

The effects of spontaneous and transplanted rat and mouse tumors on the red and white cell counts of the circulating blood have been described by Blumenthal (1), who concluded that leucocytosis is a function of the size of the tumor, whether this is of spontaneous origin or transplanted. He did not observe a significant anemia or definite leucocytosis until the tumors had reached considerable size. More recently Taylor and Pollack (12) reported that in mice planted tumors reported by Blumenthal, had caused decided anemia and leucocytosis. In the present studies no tests were made on small transplanted tumors. The tests on early induced tumors, however, showed a significant reduction in mean hemoglobin value even when the tumors were induced by very small doses (0.2 to 1.0 mgm. of benzpyrene) that initially increased the number of red cells.

Fig. 6 illustrates the reduction of hemoglobin value in rats with large transplanted tumors. Rats with J.R.S., R 92, and I.R.S. 4337 showed the greatest

decrease in hemoglobin value, while those with I.R.S. 1548 and 146 showed the least change.

The red cell counts (Fig. 7) were much reduced in rats with I.R.S. 4337, J.R.S., and R 92, but showed no reduction in rats with I.R.S. 1548 and 146. The tumors associated with the more pronounced anemia were relatively more malignant as shown by their rate of growth (Table VII) than were the two that produced less definite changes.

The increase in the percentage of polymorphonuclear neutrophil leucocytes observed in the bearers of these transplanted tumors is illustrated in Fig. 8. Parsons (10) found in mice with tumors induced by sodium 1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate a leu-

TABLE VII: AVERAGE INCREASE IN PERCENTAGE OF POLYMORPHONUCLEAR NEUTROPHIL LEUCOCYTES AND AVERAGE DAILY GAIN IN DIAMETER OF TUMORS

Tumors	Number of rats	Days from observation or inoculation	Average daily gain, mm.	Increase in polymorphonuclear leucocytes, per cent
R 92/170A	31	17–28	2.96 ± 0.09	11
I.R.S. 4337/61A	47	16–35	2.47 ± 0.03	7
R 95/66A	16	21–36	1.69 ± 0.10	18
J.R.S./366B	17	17–33	1.56 ± 0.11	21
I.R.S. 6820/21A	25	17–37	1.39 ± 0.04	15
I.R.S. 146/159A	32	31–38	1.32 ± 0.03	18
I.R.S. 1548/91A	192	16–38	1.04 ± 0.01	7
M-C, 4 to 10 mgm.	121	30.5 ± 1.0	0.90 ± 0.03	19
M-C, 1 to 3 mgm.	92	32.7 ± 1.3	0.87 ± 0.03	16
B-P, 2 to 3 mgm.	82	49.0 ± 2.2	0.63 ± 0.03	19
B-P, 0.2 to 2.0 mgm.	89	47.1 ± 2.2	0.62 ± 0.02	17

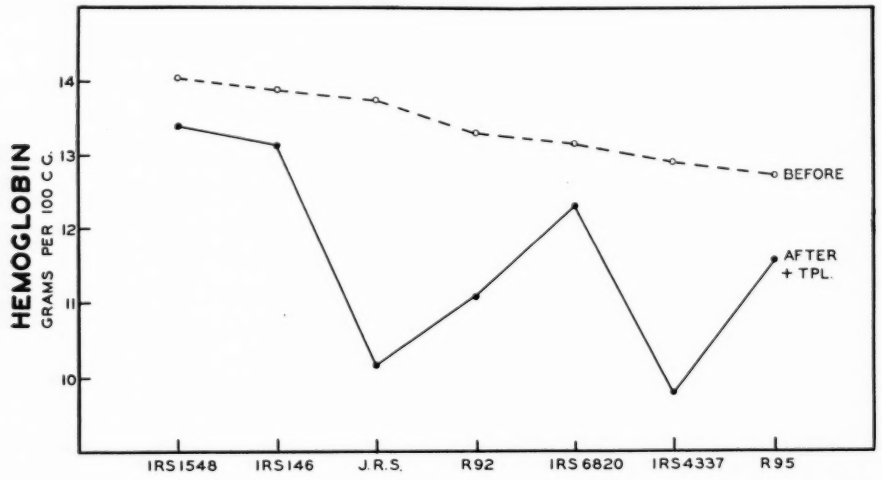

FIG. 6.—Mean hemoglobin values of rats before and after the progressive growth of a transplanted sarcoma.

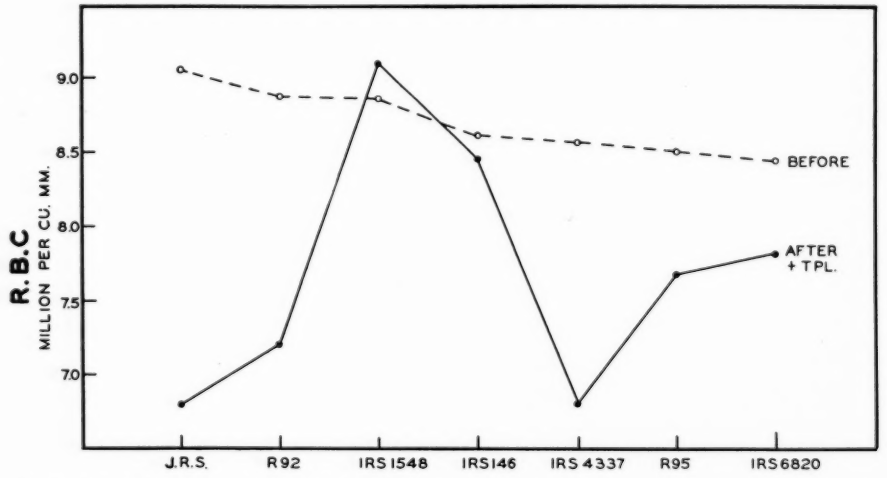

FIG. 7.—Mean red cell counts of rats before and after the progressive growth of a transplanted sarcoma.

cocytic increase more or less proportional to the rate of growth of the tumors. She observed proportional changes also in the blood of mice bearing successive grafted generations of these primary tumors. Lewis (8), however, stated that the blood picture of the tumor-bearing mouse disclosed nothing in regard to the malignancy of the straight-line tumors, but that tumors are indicated by the broken line. Obviously these increases were not related to the malignancy of the growths. The 3 transplanted tumors, R 92, I.R.S. 4337, and 1548, which showed an increase of 10 per cent or less in the percentage of polymorphonuclear leucocytes, were the two most rapidly growing and the slowest growing transplanted tumors.

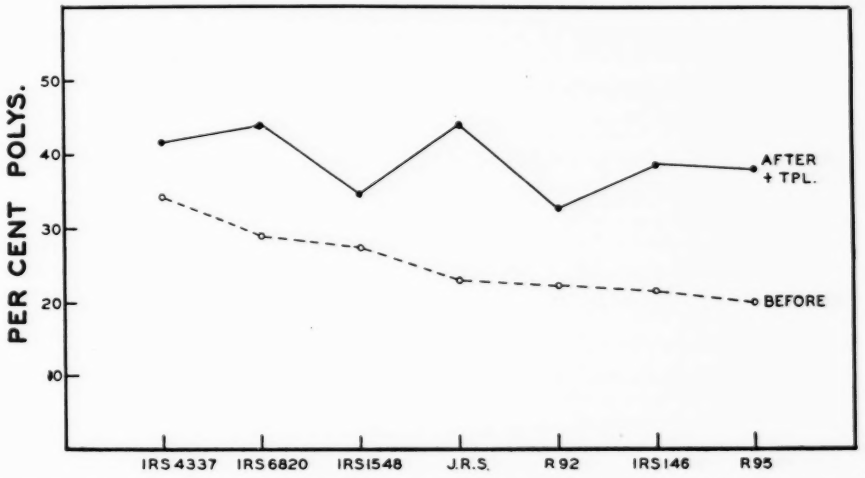

FIG. 8.—Average percentage of polymorphonuclear neutrophil leucocytes in rats before and after the growth of a transplanted sarcoma.

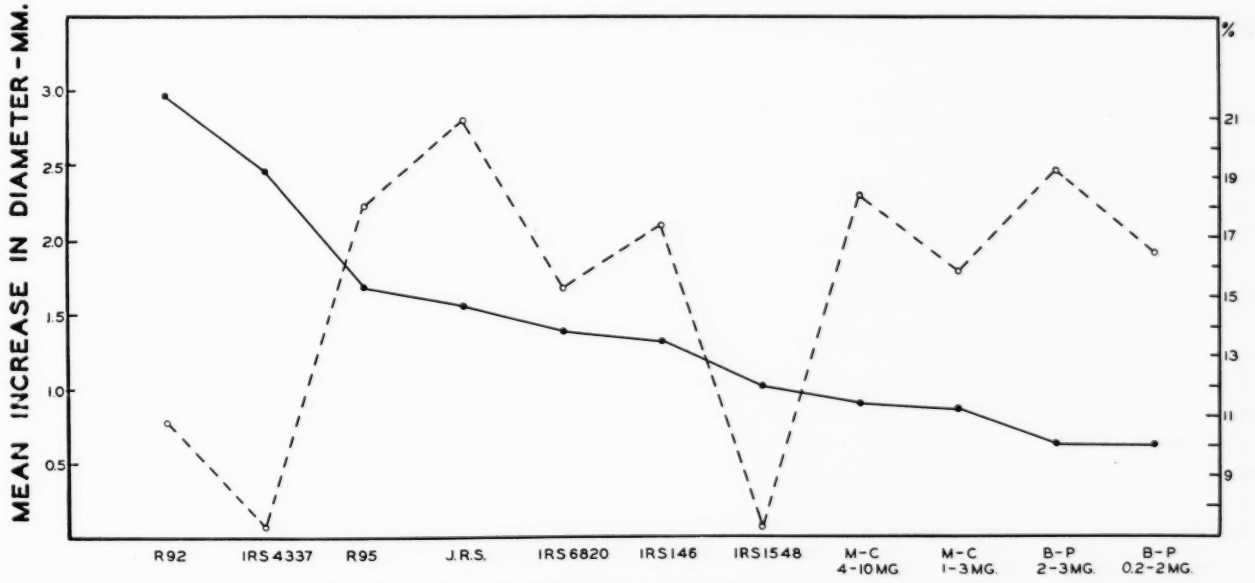

FIG. 9.—Mean daily increase in diameter of induced and transplanted sarcomas and the increase in the percentage of polymorphonuclear leucocytes shown by their hosts.

the severity of the neutrophilia in the host denoted biological differences in the tumors.

The results of the present study do not indicate a relationship between neutrophilia and malignancy. Thus in Fig. 9 the transplanted and induced tumors are arranged from left to right in the order of their average daily increase in diameter, and the respective average increases in the percentage of polymorphonuclear leucocytes occurring in the bearers of large

SUMMARY

1. The subcutaneous injection of paraffin containing methylcholanthrene or benzpyrene increased the percentage of monocytes in the peripheral blood.

2. Rats with early tumors induced by methylcholanthrene or benzpyrene showed significant decreases in their mean hemoglobin values.

3. The continued growth of the induced tumors reduced decidedly the hemoglobin values and red cell

counts of their hosts, and significantly increased the total white cell counts and the percentage of polymorphonuclear neutrophil leucocytes.

4. The progressive growth of seven transplanted sarcomas of diverse origin significantly reduced the hemoglobin value and increased the white cell count and the percentage of polymorphonuclear neutrophil leucocytes.

5. The degree of anemia observed in the hosts of induced and transplanted sarcomas appeared to be related to the malignancy of the growths, but the leucocytosis was apparently independent.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BERENBLUM, I., and SCHOENTAL, R. [Univ. of Oxford, England] THE METABOLISM OF 3,4-BENZPYRENE IN MICE AND RATS. I. THE ISOLATION OF A HYDROXY AND A QUINONE DERIVATIVE, AND A CONSIDERATION OF THEIR BIOLOGICAL SIGNIFICANCE. Cancer Research, 3: 145-150. 1943.

Injection of 3,4-benzpyrene by the intraperitoneal route in mice or rats was found to be most favorable in providing high yields of metabolic products in the feces.

By a simplified method of extraction two products were isolated from the feces: a fluorescent phenolic derivative, identical with BPX of Peacock, Chalmers, and Crowfoot, and another product, not previously described, with properties similar to synthetic 3,4-benzpyrene-5,8-quinone.

From approximate estimations it seems probable that the greater part of the benzpyrene lost from the body appears in the excreta in the form of these two isolated derivatives.

The quinone was found to be noncarcinogenic after 10 months, following subcutaneous injection of about 2 mgm. in sesame oil. Similar injections of the metabolic 8-hydroxy product (BPX) yielded a sarcoma in 1 mouse of 10.

The significance of the conversion of 3,4-benzpyrene into these two derivatives, in relation to the biological activities of the parent hydrocarbon, is discussed. While these derivatives probably play no part in the carcinogenic mechanism of the parent hydrocarbon, the arguments in favor of their being concerned with other biological activities (*e.g.* inhibition of tumor growth and estrogenic action) are suggestive, and call for further investigation.—Authors' summary.

BERENBLUM, I., CROWFOOT, D., HOLIDAY, E. R., and SCHOENTAL, R. [Univ. of Oxford, and London Hosp., England] THE METABOLISM OF 3,4-BENZPYRENE IN MICE AND RATS. II. THE IDENTIFICATION OF THE ISOLATED PRODUCTS AS 8-HYDROXY-3,4-BENZPYRENE AND 3,4-BENZPYRENE-5,8-QUINONE. Cancer Research, 3:151-158. 1943.

A study has been made of the crystallography and the fluorescence and absorption spectra of certain derivatives of the two products of 3,4-benzpyrene isolated from mouse and rat feces, and these data have been compared with similar data from comparable derivatives of synthetic compounds.

By these means it was possible to identify the red product with 3,4-benzpyrene-5,8-quinone, and to arrive at the conclusion that the fluorescent product BPX is 8-hydroxy-3,4-benzpyrene.—Authors' summary.

Hormones

HOCH-LIGETI, C. [Chester Beatty Research Inst., The Royal Cancer Hosp. (Free), London, England] STUDIES ON THE EFFECT OF CARCINOGENIC HYDROCARBONS ON THE PRODUCTION OF ANTIHORMONES. Brit. J. Exper. Path., 23:228-238. 1942.

Gordon (Cold Spring Harbor Symposium, **5**:419. 1937) showed that chorionic gonadotropin caused a greater and more prolonged increase in the weight of the ovaries in splenectomized than in normal rats. In the present experiments it was found that in black-hooded rats methylcholanthrene (colloidal solution *sub cutem*) had an effect similar to that of splenectomy. In these rats antihormone action appeared from the 15th day of treatment with gonadotropin, but if methylcholanthrene also was given the ovaries continued to increase in weight and were in the second 15 days on an average twice as heavy as those of animals receiving gonadotropin only. In some comparable groups of litter mates this difference was as much as 3-fold or 4-fold. In albino rats antihormone action was less pronounced and the effect of methylcholanthrene was doubtful. Animals treated with anthracene and gonadotropin did not differ from those receiving hormone only. The hydrocarbons alone had no significant effect on the weight of the ovaries.—E. L. K.

JONES, E. E. [Fearing Research Laboratory, Brookline, and Wellesley College, Mass.] THE EFFECT OF 2,7-DIHYDROXYNAPHTHALENE ON TUMOR INCIDENCE AND GROWTH OF THE MAMMARY GLAND IN MICE OF THE LINE A ALBINO AND C3H STRAINS. Cancer Research, 3:168-172. 1943.

Subcutaneous injections of 2,7-dihydroxynaphthalene were given to male mice of the C3H and line A albino strains and to female mice of the C3H strain to determine the effect on the growth of the mammary gland and the formation of tumors of this organ. Such injections failed to influence the incidence or the age at appearance of tumors induced by progynon B in castrated male mice of the albino strain and in normal males of the C3H strain. They also failed to affect breeding activity and the formation of spontaneous tumors in normal female mice of the C3H strain. They did not inhibit the growth of tumors nor prevent the development of multiple tumors when given to females with spontaneous tumors of palpable size. Inhibition of estrus or prolongation of diestrus was noted in young, normal females. In ovariectomized females, some evidence was noted either of a direct estrogenic effect, or of a protective effect on estrogen formed in the adrenals.

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A high mortality from scrotal hernia occurred among line A albino males that were receiving progynon B and naphthalene injections. After similar treatment, scrotal hernia did not develop in castrate albino males, and occurred less frequently and was less severe in normal C3H males than in normal albino males.

2,7-Dihydroxynaphthalene stimulated duct growth, but did not produce complete lobule-alveolar proliferation in male mammary glands. This may explain why tumor development in naphthalene-treated mice paralleled that in mice receiving progynon B alone. Line A albino mice, even after long continued injections of progynon B, failed to form numerous well developed alveoli. This is suggested as an anatomical basis for their low tumor incidence.—Author's abstract.

PALETTA, F. X., and MAX, P. F. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] INFLUENCE OF ESTRADIOL BENZOATE ON EPIDERMAL METHYLCHOLANTHRENE CARCINOGENESIS. J. Nat. Cancer Inst., 2:577-581. 1942.

Virgin female mice, 3 months old, of inbred Swiss stock were used. Forty were painted on the skin of the abdomen with 3 drops of a 0.01% solution of α-estradiol benzoate in chloroform every 4 days. Eighteen hours after each application of estrogen, these mice and 40 control animals were painted with a 0.3% solution of methylcholanthrene in benzene on a skin area about 5 mm. in diameter at the interscapular region. The calculated amount of estrogen received by each mouse at one application was 37.5 rat units. Vaginal smears indicated that the mice were continually in estrus.

In the 6th week of the experiment the first papilloma appeared in the estrogen-treated and control groups. In the 10th week benign tumors had appeared in 20% of the estrogen-treated and in 2.5% of the control mice. At the 12th week 4 squamous cell carcinomas appeared in the estrogen-treated group but none in the controls. At the 16th week 27.5% of the estrogen-treated animals and 2.5% of the controls had developed malignant tumors. At the 23rd week 82.5% of the estrogen-treated mice and 67.5% of the control mice had malignant tumors. The control animals took 1.8 weeks longer to develop malignant tumors than did the animals painted with estrogen. This difference was found to be statistically significant. No carcinomas were found on microscopic examination of mammary glands, vagina, cervix, or uterus of mice from both groups.—F. L. H.

PEARLMAN, W. H. [Clark Univ., Worcester, Mass.] STEROID EXCRETION IN CANCEROUS AND NONCANCEROUS PERSONS. I. THE 17-KETOSTEROIDS. Endocrinology, 30:270-276. 1942.

Determinations were made of the 17-ketosteroids in urine of healthy men and women, and of men and women who were hospitalized but did not have cancer. These were compared with analyses from 31 males and 33 females who had various types of cancer. It was found that noncancerous males excreted significantly greater amounts of 17-ketosteroids than did noncancerous females. This sex difference appeared to be absent in persons with cancer. Cancerous persons excreted significantly smaller amounts of 17-ketosteroids than did noncancerous persons. The decrease was most pronounced in the digitonin-precipitable hydroxyketonic fraction, which in the cancerous persons was composed entirely of isoandrosterone. The isoandrosterone, a normal constituent of human urine, was present in larger quantities in the noncancerous group and was accompanied by dehydroisoandrosterone in the males but not in the females. Androstenone-17 was isolated from noncancerous females and cancerous males. —C. A. P.

SHIMKEN, M. B., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF FOSTER NURSING ON THE INDUCTION OF MAMMARY AND TESTICULAR TUMORS IN MICE INJECTED WITH STILBESTROL. J. Nat. Cancer Inst., 2:611-622. 1942.

Litter mate male and female mice of strains C3H (high tumor), C (low tumor), and C57 black (low tumor) were used. Half of each litter were nursed by their own mother, and half foster-nursed by mothers of the other strains. Stilbestrol (4,4′-dihydroxy-α,β-diethylstilbene) was administered in large doses either in sesame oil solution or as stilbestrol-cholesterol pellets.

The results of 5 experiments in which a total of 355 male and 194 female mice was used are presented. Foster nursing of C3H males and females by C57 black or C mice reduced the incidence of mammary adenocarcinoma, and foster nursing of C males and females by C3H mice raised the incidence of mammary tumors. The milk factor was transmitted in strain C mice to the F_1 and F_2 generations without apparent dilution. Although the results obtained with mice of strain C3H and C are clear cut, those obtained with C57 mice were indefinite, probably owing to poor toleration of the estrogen.

The incidence and the latent period of primary interstitial cell tumors of the testis induced with stilbestrol in strain C mice were not affected by the presence or absence of the milk factor in the mice.

Foster nursing exerted no influence on the weight increment in mice of the 3 strains following administration of stilbestrol.

The conclusion is reached that the estrogens prepare a suitable substrate in the mamma of the mouse for the carcinogenic action of another agent, transmitted through the milk of the potentially cancerous mother or foster mother.—F. L. H.

TAYLOR, H. C., Jr., MECKE, F. E., and TWOMBLY, G. H. [Memorial Hosp., New York, N. Y.] ESTROGEN AND 17-KETOSTEROID EXCRETION IN PATIENTS WITH BREAST CARCINOMA. Cancer Research, 3:180-192. 1943.

In the first part of the study comparison is made of estrogen and androgen excretion in patients with and without breast cancer. Special subgroups were considered separately; namely, young women with normal menstrual periods, young women with amenorrhea from constitutional disease, and older women in the menopause. No characteristic differences in total estrogens, total 17-ketosteroids, or in biologically determinable androgenic substances were noted. In a few studies of male breast cancer, it was found that the excretion of 17-ketosteroids was somewhat lower than for normal men, a finding also noted in a few instances of gynecomastia.

In the second part of the study two women of the same age and with the same type of menstruation, one with breast cancer and one a normal woman, were studied for seven consecutive menstrual cycles. The first month

was accepted as a control and total estrogens and total 17-ketosteroids determined for each of about 10 consecutive 72 hour urine collections. During the next 6 months, the effects of the injection of estrone, testosterone propionate, and progesterone, injected either before or after the time of ovulation, were studied. Observation was particularly made of a susceptibility of the menstrual cycle to alterations in length and of changes in total estrogen and 17-ketosteroid excretion. Certain contrasts in the response to hormone injections were noted in the 6 months' study of this one patient with breast cancer and the one normal control. These differences in response undoubtedly reflected some physiologic differences in the two women and suggested clinical methods by which a physiologic predisposition to breast cancer might be sought in further work. In view of the fact that only two women were studied no definite conclusions could be drawn as to the specificity for breast cancer of these differences in response.—Authors' abstract.

Biochemistry and Nutrition

ABELS, J. C., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] METABOLIC STUDIES IN PATIENTS WITH CANCER OF THE GASTROINTESTINAL TRACT. XVII. THE CONJUGATION OF PHENOLS. Cancer Research, 3:177-179. 1943.

Measurements were made of the urinary excretion of free and conjugated phenols, and of glucuronates in 5 patients with hepatic cirrhosis, in 12 patients with cancer of the gastrointestinal tract, and in 12 normal persons as controls.

Patients with hepatic cirrhosis have an impaired ability to conjugate phenols. It is possible that the abnormally small amount of glucuronates excreted by these persons might be either the cause or the result of such impairment. Patients with gastrointestinal cancer, who are known to have also a considerable degree of hepatic insufficiency, have a decreased capacity to synthesize or conjugate glucuronic acid. Nevertheless, their ability to esterify phenols remains unimpaired. It is possible that the detoxification of phenols in patients with cancer of the gastrointestinal tract is not dependent necessarily upon the synthesis of glucuronic acid.—Authors' abstract.

AMERSBACH, J. C., WALTER, E. M., and COOK, E. S. [Institutum Divi Thomae, Cincinnati, Ohio, and New York Post-Graduate Med. Sch. and Hosp., New York, N. Y.] RESPIRATION OF HUMAN KERATOSES AND EPITHELIOMAS. Arch. Dermat. & Syph., 46:269-275. 1942.

A study was made of the respiration of tissues from a number of precancerous and cancerous lesions of human epithelium and of normal epithelium from the same general area. In the majority of cases respiration of the precancerous tissues and of the epitheliomas was lower than that of the normal epithelium.—H. G. W.

BELKIN, M., and STERN, K. G. [Yale Univ. Sch. of Med., New Haven, Conn.] METABOLISM OF YALE CARCINOMA 1. Cancer Research, 3:164-167. 1943.

Some of the metabolic properties of Yale carcinoma 1 have been studied. This tumor is an adenocarcinoma of the mammary gland that arose in a male mouse following the administration of estrogen.

It was found to have on the average a Q_{O_2} of -2 in air and -7 in 100% oxygen; an aerobic glycolysis of 7; and an anaerobic glycolysis of 16. The Meyerhof quotient was 1.4 to 2.2. These data suggest that the respiratory ferment in this tissue has a relatively low affinity for oxygen. The tumor exhibited a definite Pasteur effect.—Authors' abstract.

WOODARD, H. Q. [Memorial Hosp., New York, N. Y.] THE GLYCEROPHOSPHATASES OF THE RAT LIVER CANCER PRODUCED BY FEEDING *p*-DIMETHYLAMINOAZOBENZENE. Cancer Research, 3:159-163. 1943.

The alkaline β-glycerophosphatase activity of extracts of the rat liver cancer produced by the feeding of *p*-dimethylaminoazobenzene averages 10 times that of normal rat livers. The enzyme is present in the endothelial cells of the sinuses of the liver cancers, and in the nuclei of the tumor cells. The presence of increased amounts of alkaline β-glycerophosphatase in the cancers does not result in an increase in their ability to store radioactive phosphorus, when compared with normal rat livers. Neither the acid nor the alkaline β-glycerophosphatases of normal rat livers nor of rat liver cancers are inhibited by dimethyl-*p*-phenylenediamine, one of the probable metabolic products of *p*-dimethylaminoazobenzene. It is suggested that excessive amounts of alkaline β-glycerophosphatase are formed in the rat liver cancers in an attempt at compensation for some other dephosphorylating mechanism that is inactivated by *p*-dimethylaminoazobenzene or its split products.—Author's abstract.

Immunology

BARRETT, M. K. [Nat. Cancer Inst., Bethesda, Md.] ANAPHYLAXIS AND HEMORRHAGE IN TRANSPLANTED TUMORS. J. Nat. Cancer Inst., 2:625-630. 1942.

Male and female mice, 2 to 10 months old, of the inbred strains A, C, and C3H, were sensitized with a single intraperitoneal injection of 0.5 cc. of normal filtered horse serum. Seven days later these animals and comparable control mice were inoculated intracutaneously with sarcoma 37 pulp. After another 7 days both test and control animals received 0.5 cc. of horse serum intraperitoneally.

Signs of shock, consisting in erection of the hair, irregular or labored respiration, and coldness to touch, appeared in 55 of the 60 sensitized test mice as early as 15 minutes after injection of the second dose of horse serum. In every case, shock was followed within 1 hour after injection by hemorrhage of the tumor, visible through the skin, and necrosis. In the 59 control animals no signs of shock and only 1 small hemorrhage appeared.

The specific nature of the antigen was indicated by the fact that anaphylaxis and hemorrhage did not appear when 10 tumor-bearing mice that had been sensitized to horse serum were given an injection of 0.5 cc. of rabbit serum. A single intraperitoneal injection of histamine dihydrochloride in 0.5 cc. saline into strain A mice bearing 7 day old transplants of sarcoma 37 caused hemorrhage and necrosis in the tumor; 10 mgm. of the compound produced the reaction in 5 of 9 animals; 20 mgm. produced the reaction in 10 of 10 animals. Injections of Witte's peptone gave irregular reactions obtained only with highly toxic doses.

Two figures show the gross and microscopic appearance of control and test tumors.—F. L. H.

MANN, L. S., and WELKER, W. H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] FURTHER STUDIES OF SPECIFIC PRECIPITIN ANTISERUMS FOR THE PROTEIN OF CANCER TISSUE. I. Cancer Research, 3:193-195. 1943.

Precipitin antiserums for the proteins of cancer tissue were prepared in 109 rabbits. Finely ground, thoroughly washed cancer tissue was adsorbed on aluminum cream and injected into the thigh muscles of rabbits. Approximately half of the serums were free of antibodies to blood serum proteins at the end of 6 months and 85% at the end of 8 months. After the serums were free of antibodies to the blood serum proteins (in 2 to 18 months), they were titrated against autolysates of the respective tissues. Of the resultant serums 11% were considered to be potent. The precipitins for cancer tissue proteins persist for a little more than 2 months after the serums are free of antibodies to blood serum proteins.—Authors' abstract.

MANN, L. S., and WELKER, W. H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] FURTHER STUDIES OF SPECIFIC PRECIPITIN ANTISERUMS FOR THE PROTEIN OF CANCER TISSUE. II. THE APPLICATION OF IN VIVO ABSORPTION. Cancer Research, 3:196-197. 1943.

Rabbits were injected with finely ground, thoroughly washed cancer tissue and kidney tissue adsorbed on aluminum cream. Two to 3 weeks after injection the serums were titrated for antibodies to blood proteins. The animals were then injected intravenously with solutions of albumin and pseudoglobulin. Blood samples were taken at 1, 2, and 24 hour intervals. In 9 animals the blood serum protein antibodies could not be detected at the end of 1 and 2 hour intervals, but were present 24 hours after injection. Five of the rabbit serums contained antibodies for tissue proteins. The percentage of successful experiments is low, but the technic is valuable in that the time interval for obtaining a specific antiserum for tissue protein is reduced to a few weeks.—Authors' abstract.

Leukemia

MURPHY, JAS. B., and STURM, E. [Rockefeller Inst. for Med. Research, New York, N. Y.] THE EFFECT OF SODIUM PENTOBARBITAL, PARADICHLORBENZENE, AMYL ACETATE, AND SOVASOL ON INDUCED RESISTANCE TO A TRANSPLANTED LEUKEMIA OF THE RAT. Cancer Research, 3:173-175. 1943.

The repeated administration of any one of four toxic agents, sodium pentobarbital, paradichlorbenzene, amyl acetate, or sovasol, will definitely modify induced resistance to a transplanted leukemia in rats. The resistant state was induced by the injection of homologous living normal cells 12 to 14 days before the leukemia inoculation. Over 80% of the rats receiving such treatment proved resistant to the transplanted leukemia, while only 16.5% of the untreated rats were refractory. Potentially resistant rats exposed to the toxic agents showed a susceptibility to inoculation of from 57.5% to 83.5%, depending on the time and frequency of the exposures. It is noted that the four agents have leukemia-inciting properties also.—Authors' abstract.

Transplantation

PIERCE, M. [Children's Memorial Hosp., Chicago, Ill.] CULTIVATION OF HUMAN LEUKEMIC LEUKOCYTES ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICKEN EGG. Arch. Path., 34:538-545. 1942.

The chorioallantoic membrane of the developing chick embryo provides a medium in which, under suitable conditions, human leukocytes may grow for a short period. No evidence was obtained that a leukemic change could be produced in the embryo or the chick by inoculation of the membrane with human leukemic cells.—H. G. W.

ZONDEK, B., MANDL, F., SULMAN, F., BREZEZINSKI, A., and TIETZ, H. G. [Rothschild Hadassah Univ. Hosp. and Hebrew Univ., Jerusalem, Palestine] HETEROTRANSPLANTATION OF HUMAN PSEUDOMYXOMA PERITONEI INTO MICE AND RATS. Cancer Research, 3:198-205. 1943.

A pseudomucinous cystadenoma ovarii from the human subject was successfully transplanted into mice (less successfully into rats also). After 3 months the grafts showed signs of definite growth, provided implantation had been carried out intraperitoneally in the neighborhood of the kidney. In the second generation in mice there was less growth, and in the third, none at all.—Authors' abstract.

Miscellaneous

CARR, J. G. [Univ. of Edinburgh, Scotland] THE EFFECT OF SOME SUBSTANCES INFLUENCING CELL ACTIVITY UPON THE GROWTH OF THE ROUS NO. 1 SARCOMA. Brit. J. Exper. Path., 23:221-228. 1942.

The Rous sarcoma was produced by injection of filtrate into Brown Leghorn fowls of an inbred strain of low susceptibility. The tumors showed little variation in rate of growth, and were used to test the effect of various substances known to influence cell activity in mammals. No inhibition was produced by 2,4-dinitrophenol. Colchicine did not prevent the growth of established tumors, nor would it inactivate the Rous agent either *in vivo* or *in vitro*. Treatment with acenaphthene produced irregular results, both regression and stimulation of the tumors resulting.

Injection of methylcholanthrene produced a violent local reaction in birds injected with the Rous agent, with subsequent regression of established tumors and development of a Rous tumor (about 3 weeks later) at the site of injection of the hydrocarbon.—A. H.

PLAUT, A., and KOHN-SPEYER, A. C. [Beth Israel Hosp. and Coll. of Physicians and Surgeons, New York, N. Y.] THE SKIN TUNNEL. A DEVICE FOR KEEPING SUBSTANCES IN CONTACT WITH THE SKIN. Cancer Research, 3:176. 1943.

A method is described for maintaining solid or semisolid substances in contact with the skin.—Authors' abstract.

Clinical and Pathological Reports

Nervous System

DRAKE, R. L., and HELLWIG, C. A. [St. Francis Hosp., Wichita, Kans.] RETROPERITONEAL SYMPATHICOBLASTOMA WITHOUT INVOLVEMENT OF THE ADRENAL GLANDS. Arch. Path., 33:922-929. 1942.

Two cases are reported. In one, the tumor was congenital with metastasis to the brain.—H. G. W.

LIPSCOMB, W. R. [Denver, Colo.] DIAGNOSIS AND TREATMENT OF EXPANDING LESIONS OF THE CRANIAL CAVITY. Rocky Mountain M. J., 38:553-561. 1941.

Brain tumors comprise about 2% of all tumors. About 50% of this group are gliomas or primary nonencapsulated tumors arising from cerebral tissue and the remaining 50% are encapsulated. Those arising from the arachnoid compose 18%; 14% are of pituitary origin; 9% are of eighth nerve origin; and the remaining 9% are miscellaneous tumors. The symptoms, methods of diagnosis, and types of treatment of the various lesions are described and illustrative case reports presented.—R. C. R.

MASERITZ, I. H. [Johns Hopkins Hosp., Baltimore, Md.] NEUROGENIC SARCOMA. J. Bone & Joint Surg., 24:586-594. 1942.

An analysis of 216 cases from the records of the Surgical Pathological Laboratory of Johns Hopkins shows that by far the majority of the lesions arose in the extremities (149 of 192 cases). The distribution of cases was about equal in the two sexes, and the highest incidence of the disease was in the third decade of life. Irradiation was of little value, radical excision being the treatment of choice. Among 115 patients there were 20 who had lived over 5 years. Four instances of malignant transformation were observed in 36 patients with von Recklinghausen's disease. In 12 cases bones were involved.—H. G. W.

RUSSELL, W. O., and SACHS, E. [Washington Univ. Sch. of Med., St. Louis, Mo.] FIBROSARCOMA OF ARACHNOIDAL ORIGIN WITH METASTASES. REPORT OF FOUR CASES WITH NECROPSY. Arch. Path., 34:240-261. 1942.

To the 4 in the literature are added 4 more cases of malignant intracranial tumors believed to be primary from arachnoidal cells. Three patients showed remote metastases, and the fourth, invasion of blood vessels. The term "arachnoidal fibrosarcoma" is suggested for this type of tumor.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

ADAMS, W. E., STEINER, P. E., and BLOCH, R. G. [Chicago, Ill.] CARCINOMA OF THE LUNG WITH LONG CLINICAL COURSE (MALIGNANT ADENOMA). Proc. Inst. Med. Chicago, 13:296-297. 1941.

A general discussion.—R. C. R.

BEHRENTS, E. G. [Chicago, Ill.] OBTURATOR THROMBUS OF THE RIGHT PULMONARY ARTERY AND ISCHEMIC NECROSIS OF THE ENTIRE LUNG WITH HILAR BRONCHOGENIC CARCINOMA. Proc. Inst. Med. Chicago, 14:50. 1942.

A case report.—R. C. R.

CARLUCCI, G. A., and SCHLEUSSNER, R. C. [Misericordia Hosp., New York, N. Y.] PRIMARY (?) MELANOMA OF THE LUNG. A CASE REPORT. J. Thoracic Surg., 11:643-649. 1942.

Report of a case with surgical intervention but without necropsy.—H. G. W.

DAVISON, R. M., and CAUL, C. J. [Chicago, Ill.] SOME TUMORS OF THE LUNG AND MEDIASTINUM. Proc. Inst. Med. Chicago, 14:15. 1942.

The presentation of 5 cases.—R. C. R.

DORSEY, J. M. [Chicago, Ill.] DERMOID TUMORS OF THE MEDIASTINUM. Proc. Inst. Med. Chicago, 13:388. 1941.

A discussion of diagnosis and treatment.—R. C. R.

HARRINGTON, S. W. [Rochester, Minn.] INTRATHORACIC TUMORS. Proc. Inst. Med. Chicago, 14:114-115. 1942.

A general discussion of diagnosis and treatment.—R. C. R.

HORSLEY, J. S. [St. Elizabeth's Hosp., Richmond, Va.] PULSATING TUMORS OF THE ANTERIOR MEDIASTINUM. Surg., Gynec. & Obst., 75:49-53. 1942.

An unusual epithelial tumor of the sternum, with transmitted pulsation from the aorta.—H. G. W.

LEVIN, O. L., and BEHRMAN, H. T. [Beth Israel Hosp., New York, N. Y.] ACANTHOSIS NIGRICANS ASSOCIATED WITH CARCINOMA OF THE LUNG. Arch. Dermat. & Syph., 46:54-58. 1942.

Only two other cases in which acanthosis nigricans was associated with carcinoma of the lung were found in the literature.—H. G. W.

PERRONE, J. A., and LEVINSON, J. P. [Mercy Hosp., Pittsburgh, Pa.] PRIMARY CARCINOMA OF THE LUNG (REPORT OF 115 CASES, 38 AUTOPSIES, AND 77 BRONCHOSCOPIC BIOPSIES). Ann. Int. Med., 17:12-25. 1942.

A report of 115 cases, including 77 bronchoscopic diagnoses and 38 autopsies. In the 77 instances in which a diagnosis was made by bronchoscopic examination, only 3 patients were amenable to surgical treatment and 76 were dead within 3 to 18 months after the diagnosis was made. There seemed to have been a relative and absolute increase in incidence of the disease.—H. G. W.

SHAW, R. [Dallas, Tex.] PULMONARY LOBECTOMY AND PNEUMONECTOMY. Texas State J. Med., 37:529-532. 1941.

The application of surgical measures is frequently indicated in patients with pulmonary neoplasms. Three patients with adenoma of the bronchus complicated by bronchiectasis were apparently cured by excision of 1 or more of the involved lobes of the lung. Of 3 patients subjected to pneumonectomy or lobectomy for carcinoma, 2 appeared in satisfactory health 1 year or less after operation, while a recurrence in 1 responded to roentgen therapy. The possibility of achieving palliation in patients with isolated metastatic tumors of the lung by employing surgical methods is illustrated by 2 examples of temporary improvement for periods of 8 to 11 months.—M. J. E.

SWINEFORD, O., and HARKRADER, C. J., Jr. [Univ. of Virginia Med. Sch., Charlottesville, Va.] INTRATHORACIC LIPOMA; A CASE REPORT. Ann. Int. Med., 17:125-129. 1942.

This case is reported because it is the second in which asthma was the chief complaint, and because the tumor, weighing 2,310 gm., was the eighth largest of the 25 wholly intrathoracic lipomas on record.—H. G. W.

WOMACK, N. A., and GRAHAM, E. A. [Washington Univ. Sch. of Med., St. Louis, Mo.] DEVELOPMENTAL ABNORMALITIES OF THE LUNG AND BRONCHIOGENIC CARCINOMA. Arch. Path., 34:301-318. 1942.

Four cases are presented of primary cancer of the lung associated with developmental pulmonary abnormalities of

different types. The site of development of cancer may be in several foci at approximately the same time and not limited to a single cell or group of cells. This observation suggests that the preservation of embryonic potencies in maldeveloped tissues may play an important role in the development of cancer, and that susceptibility to cancer is intimately concerned with tissue differentiation. The difficulty of morphologic classification of cancer of the lung is illustrated and the lack of any significance of such a classification at the present time is emphasized—H. G. W.

Gastrointestinal Tract

CHRISTOPHER, F. [Evanston Hosp., Evanston, Ill.] HEMANGIOMA OF THE ILEUM. Proc. Inst. Med. Chicago, 14:55-56. 1942.

A case report.—R. C. R.

COOPER, K. G. [Denver, Colo.] GASTROSCOPY AS AN AID TO THE DIAGNOSIS OF STOMACH PATHOLOGY. Rocky Mountain M. J., 39:118-124. 1942.

Gastroscopy, as an aid to gastric differential diagnosis, and its contraindications are discussed. Illustrations of various lesions with discussions are included.—R. C. R.

DANGREMOND, G. [Chicago, Ill.] OBSTRUCTIVE CARCINOID OF THE ILEUM. Proc. Inst. Med. Chicago, 13:330. 1941.

General discussion of clinical and pathological characteristics with case reports.—R. C. R.

DAVID, V. C., and GILCHRIST, R. K. [Chicago, Ill.] EXTENSION OF THE BORDERLINE OF OPERABILITY IN CANCER OF THE RECTUM. Proc. Inst. Med. Chicago, 14: 184-185. 1942.

In spite of infiltration and metastases in cases of carcinoma of the rectum, many rather extensive one-stage abdominoperineal resections have been done by the authors with apparently good results.—R. C. R.

PHEMISTER, D. B. [Chicago, Ill.] TRANSTHORACIC RESECTION OF CARCINOMA OF THE ESOPHAGUS AND OF THE PROXIMAL PORTION OF THE STOMACH. Proc. Inst. Med. Chicago, 14:145-146. 1942.

Progress in the transthoracic approach to esophageal tumors is discussed and cases reported.—R. C. R.

SHAPIRO, N., SCHIFF, L., MAHER, M. M., and ZINNINGER, M. M. [Univ. of Cincinnati Med. Sch. and Cincinnati General Hosp., Cincinnati, Ohio] SOME OBSERVATIONS ON ATROPHIC GASTRITIS AND GASTRIC CANCER. J. Nat. Cancer Inst., 2:583-588. 1942.

The paper reports part of an attempt to throw light on the relationship, if any, between gastritis and gastric cancer, by the repeated examination of patients with atrophic gastritis in order to detect the early development of malignant growth.

In a study of 35 cases of proved cancer of the stomach with adequate microscopic sections, 28 patients showed microscopic evidence of atrophic gastritis and only 10 showed gastritis gastroscopically. Gastritis occurred more frequently in patients having a long history of digestive disturbance. No correlation existed between gastric acidity and the site of cancer. Achlorhydria and anemia of a hypochromic microcytic or normocytic type occurred more often in patients with atrophic gastritis.—F. L. H.

Bone and Bone Marrow

ANDERSON, C. E., and SAUNDERS, J. B. deC. M. [Univ. of California Med. Sch., San Francisco, Calif.] PRIMARY ADAMANTINOMA OF THE ULNA. Surg., Gynec. & Obst., 75: 351-356. 1942.

The first case is reported of adamantinoma of a long bone other than the tibia.—H. G. W.

BROWN, W. O. [Chicago, Ill.] LIPOSARCOMA OF BONE. Proc. Inst. Med. Chicago, 13:330-331. 1941.

Two case reports and discussion.—R. C. R.

CHURG, J., and GORDON, A. J. [Mt. Sinai Hosp., New York, N. Y.] MULTIPLE MYELOMA WITH UNUSUAL VISCERAL INVOLVEMENT. Arch. Path., 34:546-556. 1942.

A case of multiple myeloma is described in which there was unusually extensive involvement of the spleen and, to a lesser degree, of the liver and the lymph nodes. The probable origin of myeloma cells from the reticulum of the bone marrow is stressed. It is suggested that the relationship between multiple myeloma and myeloid leukemia may be analogous to that between lymphosarcomatosis and lymphatic leukemia.—H. G. W.

CORAY, Q. B. [Salt Lake City, Utah] GIANT CELL TUMOR OF THE SPINE. Rocky Mountain M. J., 38:631-632. 1941.

Two case reports.—R. C. R.

DOCKERTY, M. B., and MEYERDING, H. W. [Mayo Clinic, Rochester, Minn.] ADAMANTINOMA OF THE TIBIA. REPORT OF TWO NEW CASES. J.A.M.A., 119:932-937. 1942.

To the 14 found in the literature, the authors add 2 cases of primary adamantinoma of the tibia. These tumors are slow growing and trauma is a probable etiologic factor. Enamel has not been found in the tibial tumors, and the evidence proves that they are merely modified squamous cell growths that vary considerably in their differentiation into ameloblasts.—H. G. W.

JAFFE, H. L., and LICHTENSTEIN, L. [Hosp. for Joint Diseases, New York, N. Y.] NON-OSTEOGENIC FIBROMA OF BONE. Am. J. Path., 18:205-221. 1942.

Non-osteogenic fibroma of bone is the name given by the authors to an entity which has been interpreted in many different ways; for example, as variant forms of giant cell tumor of bone or variant forms of localized osteitis fibrosa. In the opinion of the authors, non-osteogenic fibroma bears no relation to either osteitis fibrosa or giant cell tumor, but represents a benign tumor formed from matured marrow connective tissue cells.

Observations were made upon 10 new cases. The lesion was found in both boys and girls between 6 and 21 years of age, who presented no characteristic clinical story. The tumor was located in the shaft near one end of tibia, fibula, femur, or ulna; by roentgen examination it was sharply delimited and seen to bulge out on one side of the shaft. In the gross, the lesion was yellow-brown and fibrous; microscopically, it consisted of whorled bundles of spindle-shaped connective tissue cells loosely interspersed with small multinuclear giant cells, without osseous trabeculae. In half the cases there were areas containing foam cells, but the authors feel that this should not lead to confusion with lipoid granulomatosis (Hand-Schüller-Christian's disease). Treatment consists only of thorough curettement of the affected area.—H. B.